

SERO-EPIDEMIOLOGICAL AND HAEMATOLOGICAL STUDIES ON TOXOPLASMOSIS IN CATS, DOGS AND THEIR OWNERS IN LUCKNOW - INDIA

A.Kumar¹, P. Rai² and Neeraj Sinha³

¹Director, Pet Aids Centre, Indira Nagar, Lucknow; ²Former Professor & H.O.D., Medicine, COVAS, Mathura; ³Scientist-F Division of Toxicology, Central Drug Research Institute, Lucknow, India.

Infection with *Toxoplasma gondii* is one of the most common parasitic infections of human being and other warm-blooded animals. It has been found worldwide from Alaska to Australia. Public health organizations repeatedly encourage the collection of accurate data about *T. gondii* in animals and humans due to its medical importance as a major source of parasitic zoonosis. For these reasons, epidemiological updates on toxoplasmosis in pet animals are strongly advised also to plan control strategies. In the present paper, seroprevalence data on *T. gondii* that have been recorded in Dog, Cats their owners and staff of Dog Clinics/ Vet. Hospitals from different localities of Lucknow Town (State Capital of U.P. -India) over the last one decade are reviewed, showing the level of exposure of Dogs, Cats, Pet Owners and Clinical Staffs to this parasite.

Key words: *Toxoplasma gondii*, Epidemiology, CT (Congenital Toxoplasmosis) Dogs, Cats, Lucknow

Introduction:

Infection with the protozoan parasite *Toxoplasma gondii* occurs worldwide. Cats and other felids are the only definitive hosts in which sexual reproduction occurs to produce infective oocysts. Warm-blooded animals, including humans, are intermediate hosts that harbour tissue cysts in their bodies (Scala *et al.*, 2008). Active infection is usually acquired by oral route and presumably results in lifelong parasite colonization and specific antibodies production. Consequently, serological IgG testing offers the opportunity to measure specific individual protection and public health impact of toxoplasmosis (cross-sectional study). In general, seroprevalence displays highest in the southern America and Europe, in Central America and sub-Saharan Africa, lowest in the far East. In the last decades a negative trend in infection rate has been demonstrated in many European countries and U.S.A. and attributed to improvement in general lifestyle hygiene and in food chain storage and transport, thus potentially biasing health impact evaluation (Forsgren *et al.*, 1991). As, with few exceptions, acute phase of toxoplasmosis in otherwise healthy humans occurs in a self-limited subclinical or mild form it can only be detected by serological screening for *T. gondii* antibodies. The most common clinical picture consists of isolated cervical or occipital lymphadenopathy staying for 4 to 6 weeks. Chorioretinitis leading to permanent visual loss in nearly 25% of patients can complicate congenital and postnatally acquired disease as a result of acute infection or reactivation, with differences in prevalence according to different settings (Jones *et al.*, 2006; McLeod *et al.*, 2006). In immunocompromised host complications such as myocarditis, polymyositis, hepatitis, or encephalitis may arise. Primary toxoplasmosis on gestation can be transmitted through the placenta to overall 30% of the foetus with serious permanent, even fatal consequences, such as death *in utero*, hydrocephalus, microcephalus, chorioretinitis, and intracranial calcifications. Non specific symptoms mimicking congenital toxoplasmosis (CT) with other pathogens, such as hepato-splenomegaly, purpura, jaundice and intra-uterine growth retardation, have been described (McAuley *et al.*, 1994). Furthermore, over Europe preterm birth, low birth weight and small for gestational age were not confirmed significantly different in infected babies compared with uninfected, and severe generalized onset was found uncommon in a screening care setting (Freeman *et al.*, 2005; Gras *et al.*, 2005).

Identification of locally prevalent risk factors is critic for health education, and more in general for policy. Depending on lack of tests for distinguishing infection from environmentally robust stages (oocysts transmitted by soil contamination with cat faeces) from tissue stage (cysts ingested by infected meat), knowledge on the relative importance of different sources were derived from epidemiological surveys comparing risk factors distribution in infected and uninfected individuals. Unfortunately, questionnaire survey are biased by recall bias and results must be adjusted for main confounders, such as age, education level, parity, gestational age at testing and at interviewing, making the analysis of results and conclusions quite complicated. An Italian prospective risk factor

study on pregnant women found that eating cured pork or raw meat at least once a month was associated with a threefold higher risk of *T. gondii* infection (Buffolano *et al.*, 1996). A European multicentre case-control study showed that contact with raw or undercooked meat, as well as contact with soil were independent risk factors for *T. gondii* sero-conversion on gestation (Cook *et al.*, 2000). The population attributable fraction demonstrated that 30-63% of sero-conversions were due to the consumption of undercooked or cured beef, lamb, or other source meat products and 6-17% was a result of soil contact. None of multiple different cat exposures (specifically, having a cat or kitten at home, cleaning the litter box, and owning a cat that hunts) were found to be significant risk factors. Therefore, control of *T. gondii* infections should include provision of *T. gondii* free meat products. The organotropism of *T. gondii* and the number of tissue cysts produced in a certain organ vary with the intermediate host species. Although *T. gondii* has never been isolated from edible beef in Europe or North America, beef has been found source of human infection in questionnaire surveys. Adulteration of beef by different cheaper meat species and non-skeletal muscle (heart, diaphragm, tongue) in grocery stores can't be excluded, especially in the case of minced meat such as in hamburger and sausages. Surprisingly, pork previously identified as a main risk factor in Norway and Italy was not reported as a route for infection in this study, possibly because the presence of tissue cyst in pork has decreased, and/ or pregnant women are most aware of this specific risk. Question arose on type of cooking, and freezing and/or curing methods safety. Sporulated oocysts are very resistant to environmental conditions and to disinfectants; however, they are killed within 1-2 min by heating to 55-60°C and the risk of infection is reduced by deep-freezing meat (-12°C or lower) before cooking (Hill *et al.*, 2006). Tissue cysts are also killed by gamma irradiation at a dose of 1.0 kGy, but irradiation of meat has not been approved in the EU. Recently, high pressure processing at 300 MPa or higher has been shown to inactivate tissue cysts of *T. gondii* under laboratory conditions. Travel outside Europe, USA and Canada was also a risk factor for infection. In Cook's study (Cook *et al.*, 2000) no risk factor was identified in a third of the cases. Access for cat to outdoor environment, and feeding cats with leftovers or with raw viscera were shown risk factors for human infection in Mexico and Brazil (Galvan Ramirez *et al.*, 1999).

Rain and surface water may transport infectious oocysts into drinking water supplies and irrigation waters. Climate play an indirect role in allowing the more (in the case of moist and hot climate) or less (in the case of dry and cold climate) survival of oocysts in the environment. In Brazil, drinking unfiltered water was demonstrated a risk factor (de Moura *et al.*, 2006). The largest and best documented water associated outbreak of acute toxoplasmosis in humans occurred in 110 individuals in Vancouver, Canada, in 1995 (Aramini *et al.*, 1999). *T. gondii* infection and agents thereof have to be reported by EU Member States according to their epidemiological situation (Directive 2003/99/EC); furthermore, nor humans nor animal nor food-related representative data were available on 2005. A recent questionnaire survey on programmes for the epidemiological surveillance of CT has shown 12 out of 28 responding countries did not have a surveillance system. Only four of them operate a specific surveillance (Denmark, France, Germany and Italy) (Benard *et al.*, 2008; Gollub *et al.*, 2008). Very recently, differences have been shown in the incidence of visual sequelae of CT between EU and South American patients (Gilbert *et al.*, 2008); moreover, polymorphisms at COL2A1 and ABCA4 locus have been associated with brain and ocular disease (Jamieson *et al.*, 2008).

Material & Methods:

Toxoplasmosis is a parasitic zoonosis with worldwide distribution, caused by *Toxoplasma gondii* and is very common in cats, dogs and human. Keeping in view the zoonotic importance of the disease, the current study was conducted to find out the epidemiological status of toxoplasmosis in cat, dog and human population in Lucknow city of India and to determine the possibility of transmission of toxoplasmosis from cats and dogs to their owners. For this purpose sera samples from 50 cats (n=25 domestic, n=25 stray) 100 dogs (n=50 domestic, n=50 stray) and 150 human (n=25 cat owners, n=50 dog owners, n=25 people having no contacts either with cats or dogs, n=30 employees of the various Dog Clinics and Veterinary Hospitals i.e. working at these institutions were analyzed by using Latex Agglutination Test (LAT) to determine the anti-toxoplasma antibodies at serum dilutions of 1:16, 1:64, 1:128 and 1:256.

Results and Discussion:

Overall 56% cats were sero-positive for anti-toxoplasma antibodies. Stray cats had the high prevalence (64%) followed by domestic cats (48%). The highest prevalence (71%) was detected in cat in the 7 year or above age group. The sero-positivity percentage of toxoplasmosis was highest in local breeds of the cats (64%). Furthermore the domestic cats, which had wandering habits, had higher seropositivity (62%) than the cats, which had not these habits (41%). Overall 39% dogs were sero-positive for anti-toxoplasma antibodies. Stray dogs had the high prevalence (50%) than the domestic dogs (28%). The highest prevalence of toxoplasmosis (45.9%) was recorded in dogs of age group of >1-3 years. Dogs having access to house as well as yard has the highest prevalence (40%) following the dogs having access only to yard (25%) and the dogs kept strictly at homes had the lowest prevalence (16%). Overall 22% human were sero-positive for anti-toxoplasma antibodies. The highest seropositivity was observed in cat owners (32%) followed by dog owners (26%), Dog Clinics/ Vet. Hosp. employees (20%) and the lowest sero-positivity (14%) was observed in people having no contact with dogs and cats. There was decrease in haemoglobin level of cats, dogs and human positive for anti-toxoplasma antibodies.

Conclusion:

In conclusion, the present study shows that seroprevalence data on toxoplasmosis in dogs, cats and human beings are not uniformly distributed along the whole Lucknow. In addition, these data, although revealing a *scenario* at risk from a zootechnical and sanitary point of views, represent a situation highly heterogeneous, probably due to the different sampling and laboratory techniques utilized. Thus, a coordinated national-scale survey on toxoplasmosis in dogs, cats and humans - based on homogeneous sampling and laboratory techniques - is strongly needed, in order to better assess the actual epidemiological situation of this under-estimated zoonosis in dogs and cats and to clarify factors that influence its presence and distribution.

References:

- Aramini JJ, Stephen C, Dubey JP, Engelstoft C, Schwantje H, Ribble CS. (1999). Potential contamination of drinking water with *Toxoplasma gondii* oocysts. *Epidemiol Infect.* 122:305-15.
- Bénard A, Petersen E, Salamon R, Chêne G, Gilbert R, Salmi LR; European Toxo Prevention Study Group (EUROTOXO) (2008). Survey of European programmes for the epidemiological surveillance of congenital toxoplasmosis. *Euro Surveill.* 13: 18834.
- Buffolano W, Gilbert RE, Holland FJ, Fratta D, Palumbo F, Ades AE. (1996). Risk factors for recent toxoplasma infection in pregnant women in Naples. *Epidemiol Infect.* 116: 347-51.
- Cook AJ, Gilbert RE, Buffolano W, Zufferey J, Petersen E, Jenum PA, Foulon W, Semprini AE, Dunn DT. (2000). Sources of *Toxoplasma* infection in pregnant women: European Multicentre case-control study. *European Research Network on Congenital oxoplasmosis. BMJ.* 321:142-147.
- de Moura L, Bahia-Oliveira LM, Wada MY, Jones JL, Tuboi SH, Carmo EH, Ramalho WM, Camargo NJ, Trevisan R, Graça RM, da Silva AJ, Moura I, Dubey JP, Garrett DO. (2006). Waterborne toxoplasmosis, Brazil, from field to gene. *Emerg Infect Dis.* 12: 326-9.
- Freeman K, Oakley L, Pollak A, Buffolano W, Petersen E, Semprini AE, Salt A, Gilbert R; European Multicentre Study on Congenital Toxoplasmosis. (2005). Association between congenital toxoplasmosis and preterm birth, low birthweight and small for gestational age birth. *BJOG* 112: 31-7.
- Forsgren M, Gille E, Ljungström I, Nokes DJ. (1991). *Toxoplasma gondii* antibodies in pregnant women in Stockholm in 1969, 1979, and 1987. *Lancet* 337: 1413-4.
- Gilbert RE, Freeman K, Lago EG, Bahia-Oliveira LMG, Tan HK, Wallon M, Buffolano W, Stanford MR, Petersen E for the European Multicentre Study on Congenital Toxoplasmosis (EMSCOT) (2008) Ocular sequelae of congenital toxoplasmosis in Brazil compared with Europe. *PLoS Neglected Tropical Diseases.*
- Gollub EL, Leroy V, Gilbert R, Chêne G, Wallon M; European Toxoprevention Study Group (EUROTOXO) (2008). Effectiveness of health education on *Toxoplasma*-related knowledge,

- behaviour, and risk of seroconversion in pregnancy. *Eur J Obstet Gynecol Reproduc.* 136:137-45.
- Gras L, Wallon M, Pollak A, Cortina-Borja M, Evengard B, Hayde M, Petersen E, Gilbert R; European Multicenter Study on Congenital Toxoplasmosis. (2005). Association between Prenatal treatment and clinical manifestations of congenital toxoplasmosis in infancy: a cohort study in 13 European centres. *Acta Paediatr.* 94: 1721-31.
- Hill DE, Benedetto SM, Coss C, McCrary JL, Fournet VM, Dubey JP. (2006). Effects of time and temperature on the viability of *Toxoplasma gondii* tissue cysts in enhanced pork loin. *J. Food Prot.* 69: 1961-5.
- Jamieson SE, de Roubaix LA, Kuan Tan H, Cortina-Borja M, Mui E, Cordell HJ, Mack D, Kirisitsand M, Miller EN, Peacock C, Hargrave A, Boyer K, Bardo D, Bessieres MH, Buffolano W, Ferret N, Franck J, Heydemann P, Kieffer F, Meier P, Mets M, Nowakowska DE, Patel D, Paul M, Peyron F, Stray-Pedersen B, Remington J, Swisher CN, Thulliez P, Wallon M, Petersen E, McLeod R, Gilbert RE, and Blackwell JM. (2008). COL2A1 and ABCA4 are epigenetically modified and associated with congenital toxoplasmosis, www.plosone..
- Jones JL, Muccioli C, Belfort R Jr, Holland GN, Roberts JM, Silveira C. (2006). Recently acquired *Toxoplasma gondii* infection, Brazil. *Emerg Infect Dis.* 12: 582-7.
- McAuley J, Boyer KM, Patel D, Mets M, Swisher C, Roizen N, Wolters C, Stein L, Stein M, Schey W, *et al.* (1994). Early and longitudinal evaluations of treated infants and children And untreated historical patients with congenital toxoplasmosis: the Chicago collaborative Treatment Trial. *Clin Infect Dis.* 18: 38-72.
- McLeod R, Boyer K, Karrison T, Kasza K, Swisher C, Roizen N, Jalbrzikowski J, Remington J, Heydemann P, Noble AG, Mets M, Holfels E, Withers S, Latkany P, Meier P; Toxoplasmosis Study Group. (2006). Outcome of treatment for congenital toxoplasmosis, 1981-2004: the National Collaborative Chicago- Based, Congenital Toxoplasmosis Study. *Clin Infect Dis.* 42: 1383-94.
- Scala A, Giobbe M, Mula PP, Pipia AP, Sanna-Coccone G, Firinu A, Varcasia A, Marrosu R, Garippa G (2008). Toxoplasmosi dei suidi in Sardegna: indagine sieroepidemiologica. *Parassitologia*:50.