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TOXOPLASMA GONDII ANTIBODY PREVELANCE IN LIBYAN EPILEPTIC CHILDREN. A CONTOLLED STUDY.

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Homeland is the place we love, it is where our feet may leave, but our hearts remain in it.

(Oliver Wendell Holmes)

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ABBREVIATIONS

°C	Degree Celsius
%	Percentage
<	Less than
>	More than
CT	Computed tomography
MRI	Magnetic resonance imaging
IgG	Immunoglobulin G
CNS	Central Nervous System
CSF	Cerebrospinal fluid
CI	Confidence interval
OR	Odds ratio
e.g.	For example
T. gondii	Toxoplasma gondii

ABSTRACT
RESUMEN

1. ABSTRACT/RESUMEN

1.1. ABSTRACT

1.1.1. Background

The parasitic infection toxoplasmosis has been suggested as a possible etiologic agent of epilepsy of unknown origin and other neuropsychiatric diseases. In recent years there is increased evidence of the relation of *T. gondii* acquired infection in non-inmunodeficient subjects with epilepsy. But studies in children are scarce and with contradictory results. A case-control study has been conducted to investigate the hypothesis that Libyan children with cryptogenic epilepsy have a higher prevalence of seropositivity to *T. gondii* than the control population.

1.1.2. Objectives:

The main objective of the study was to evaluate the relationship between cryptogenic epilepsy and toxoplasmosis in Libyan children. The secondary objectives were to assess the toxoplasmosis relationships with seizure types and seizure control and to identify behavioural risk factors for acquired toxoplasmosis in Libyan children.

1.1.3. Methods

Study Design: hospital-based, case-controlled study.

Setting: National Centre for Treatment of Epilepsy and Tripoli Medical Centre, Tripoli, Libya.

Subjects: The target population were cryptogenic epilepsy from (National Epileptic Centre). Control groups (symptomatic epilepsy and non-epileptic children) were taken from National Epileptic Centre and Tripoli Medical Centre.

A total of 298 children were recruited over a one-year period. The group of cases were 92 children with suspected cryptogenic epilepsy. Two control groups included 56 children with symptomatic epilepsy and 150 children without epilepsy. The ages ranged

from 6 to 17 years. The children were surveyed with a questionnaire given to each child's parent or legal guardian. Blood samples were studied for the presence of IgG anti-*Toxoplasma* antibodies.

1.1.4. Results:

A total of 298 children were included with mean age 11.14 ± 3.25 years (range, 6– 17.5 years). Were classified in three groups as suspected cryptogenic epilepsy (case, n = 92; 54 male and 38 female) and two controls; symptomatic epilepsy (n = 56; 35 male and 21 female) and non-epileptic (n = 150; 81 male and 69 female) were enrolled in the study. Residence was urban, suburban and rural in 29%, 32% and 39% respectively. The seropositivity rate for anti-Toxoplasma IgG antibodies among suspected cryptogenic epilepsy patients (35.2%) was not statistically different of symptomatic epilepsy (29.4%) and non-epileptic children (29%). There were a significant difference between mean of anti-Toxoplasma (IgG) titters in cryptogenic epilepsy (24.7±8.4 IU/mL) and those in symptomatic epilepsy (15.3±5.8 IU/mL) and non-epileptic subjects (16.1±4 IU/mL) ($p< 0.05$). High anti-*T. gondii* IgG titters (>75 IU/mL) were significantly more frequent in cryptogenic than symptomatic epilepsy children and non-epileptic control (17.4% versus 6.7%, 6.6%; $p< 0.05$).

On the other hand, prevalence of T. gondiii infection was higher in children with focal seizures than in those with generalized seizures and non-epileptic controls (47.1%, 25% and 29.4% respectively; $p<0.01$). Logistic regression analysis; confirmed that focal seizures were only epilepsy subtype positively associated with toxoplasmosis (OR = 2.32; 95% CI: 1.25–4.32; $p <0.01$).

Behavioral factors related with *T. gondii* infection, were identified by bivariate and multivariate analysis. Variables related to increased risk for toxoplamosis were eating raw meat (OR 3.16), eating raw vegetables (OR 4.02), contact with soil (OR 3.46), house cat and neighbourhood cat (OR 2.8 and 4.12 respectively) (all $p<0.01$). Drinking water from wells and rain deposits was negatively related with toxoplasmosis (OR 0.37).

1.1.5. Conclusions

There were no significant differences in the prevalence of anti-*T. gondii* antibodies among patients with cryptogenic epilepsy and controls. However, patients with cryptogenic epilepsy had higher antibody titers than the other groups and higher frequency of subjects with high titers of anti-Toxoplasma IgG. These latter findings may suggest a certain relationship between *T. gondii* infection and cryptogenic epilepsy.

A higher prevalence of anti-Toxoplasma antibodies was found in children with focal epilepsy than in patients with generalized epilepsy and non-epileptic controls, suggesting a relationship between focal epilepsy and acquired toxoplasmosis. *T. gondii* infection was not found to influence the control of epilepsy. Eating undercooked meat, raw vegetables, contact with the soil and contact with cats were related to the acquisition of toxoplasmosis.

Based on these results, it can be concluded that *Toxoplasma gondii* infection may play some pathogenic role in focal epilepsy and perhaps also in cryptogenic epilepsy. The impact of toxoplasmosis-related epilepsy should be evaluated and taken into account in order to design optimal preventive measures against T. Gondii infection in Libya.

1.1.6. Key words

Toxoplasmosis, *Toxoplasma gondii*, epilepsy, cryptogenic epilepsy, focal seizures, seroprevalence, risk factors, antibody titers, IgG, children, adolescents, Libya, Libyan epileptic children, Tripoli, North Africa.

1.2. RESUMEN

1.2.1. Antecedentes

La toxoplasmosis adquirida ha sido sugerida como una posible etiología de la epilepsia de origen desconocido y otras enfermedades neuropsiquiátricas. En los últimos años se incrementa la evidencia de la relación entre la infección adquirida por T. gondii en sujetos no inmunodeficientes con la epilepsia. No obstante, los estudios en niños son escasos y con resultados contradictorios. Se ha realizado un estudio caso-control para investigar la hipótesis de que los niños libios con epilepsia criptogénica tienen una mayor prevalencia de seropositividad frente a *T. gondii* que la población de control.

1.2.2. Objetivos:

El objetivo principal del estudio fue evaluar la posible relación entre la epilepsia criptogénica y la toxoplasmosis en niños libios. Los objetivos secundarios fueron evaluar las relaciones de toxoplasmosis con los tipos de convulsiones y el control de las crisis e identificar los comportamientos que constituían factores de riesgo para la toxoplasmosis adquirida en niños libios.

1.2.3. Métodos

Diseño del estudio: estudio clínico basado en un caso controlado.

Lugar: National Centre for Treatment of Epilepsy and Tripoli Medical Centre, Tripoli, Libya.

Sujetos: La población diana fue pacientes pediátricos con sospecha de epilepsia criptogénica (National Centre for Treatment of Epilepsy). Los grupos de control (epilepsia sintomática y niños no epilépticos) fueron recogidos en el National Centre for Treatment of Epilepsy y el Tripoli Medical Centre.

Un total de 298 niños fueron reclutados en un período de un año. El grupo de casos fueron 92 niños con sospecha de epilepsia criptogénica. Los dos grupos de control incluyeron a 56 niños con epilepsia sintomática y a 150 niños sin epilepsia. Las edades comprendían de 6 a 17 años. Los niños fueron entrevistados con un cuestionario

entregado a los padres de cada niño o a su tutor legal. Se estudiaron muestras de sangre para detectar la presencia de anticuerpos IgG anti-Toxoplasma.

1.2.4. Resultados

Se incluyeron 298 niños con edad media de 11,14 ± 3,25 años (rango, 6- 17,5 años). Se clasificaron en tres grupos, pacientes con sospecha de epilepsia criptogénica (casos, n = 92; 54 hombres y 38 mujeres) y dos grupos de control de niños con epiilepsia sintomática (n = 56, 35 hombres y 21 mujeres) y no epilépticos (n = 150, 81 hombres y 69 mujeres). El lugar de residencia fue en zona urbana, suburbana y rural en el 29%, 32% y 39% respectivamente.

La tasa de seropositividad de los anticuerpos anti-Toxoplasma IgG entre los pacientes con sospecha de epilepsia criptogénica (35,2%) no fue estadísticamente diferente de los pacientes con epilepsia sintomática (29,4%) y los controles no epilépticos (29,4%). Hubo diferencias significativas entre los títulos de IgG anti-Toxoplasma entre los pacientes con epilepsia criptogénica (24,7 ± 8,4 UI / ml) y los que presentaban epilepsia sintomática (15,3 ± 5,8 UI/mL) y los controles no epilépticos (16,1 ± 4 UI/mL) ($p< 0,05$). Los títulos altos de IgG anti-*T. Gondii* (>75 UI/ml) fueron significativamente más frecuentes en niños con epilepsia criptogénica que en niños con epilepsía sintomática y en controles no epilépticos (17,4% versus 6,7% y 6,6%, $p <0,05$).

Por otro lado, la prevalencia de infección por *T. gondiii* fue mayor en niños con convulsiones focales que en aquellos con convulsiones generalizadas y controles no epilépticos (47,1%, 25% y 29,4% respectivamente, $p <0,01$). El análisis de regresión logística confirmó que las convulsiones focales eran el único subtipo de epilepsia positivamente asociado con toxoplasmosis (OR = 2,32; IC 95%: 1,25-4,32; $p <0,01$).

Los factores conductuales relacionados con la infección por T. gondii, se identificaron por análisis bivariado y multivariante. Las variables relacionadas con un mayor riesgo de toxoplasmosis fueron comer carne cruda o poco cocinada (OR 3,16), comer verduras crudas (OR 4,02), contacto con suelo (OR 3,46), gato doméstico y gato en el vecindario (OR 2,8 y 4,12 respectivamente) ($p <0,01$). Obtener el agua para beber de pozos y depósitos de lluvia se relacionó negativamente con la toxoplasmosis (OR 0,37).

1.2.5. Conclusiones

No se encontraron diferencias significativas en la prevalencia de anticuerpos anti *T. gondii* entre los pacientes con epilepsia criptogénica y los controles. Sin embargo, los pacientes con epilepsia criptogénica tuvieron títulos de anticuerpos más elevados que los otros grupos y mayor frecuencia de sujetos con títulos altos de IgG anti-Toxoplasma. Estos últimos hallazgos pueden sugerir una cierta relación entre la infección por *T. gondii* y la epilepsia criptogénica.

Se encontró una mayor prevalencia de anticuerpos anti toxoplasma en los niños con epilepsia focal que en los pacientes con epilepsia generalizada y los controles no epilépticos, lo que sugiere una relación entre la epilepsia focal y la toxoplasmosis adquirida. No se encontró que la infección por *T. gondii* influyera en el control de la epilepsia. El comer carne poco cocinada, comer vegetales crudos, el contacto con el suelo y el contacto con gatos se relacionaron con la adquisición de toxoplasmosis.

Basándose en estos resultados, se puede concluir que la infección por *Toxoplasma gondii* puede desempeñar un papel etiopatogénico en la epilepsia focal y quizás también en la criptogénica. El impacto de la epilepsia relacionada con la toxoplasmosis debe ser evaluado y tomado en cuenta para diseñar medidas preventivas óptimas contra la infección por *T. Gondii* en Libia.

1.2.6. Palabras clave

Toxoplasmosis, Toxoplasma gondii, epilepsia, epilepsia criptogénica, convulsiones focales, seroprevalencia, factores de riesgo, títulos de anticuerpos, IgG, niños, adolescentes, Libia, niños epilépticos libios, Trípoli, África del Norte.

INTRODUCTION AND BACKGROUND

2. INTRODUCTION AND BACKGROUND

2.1. Background and Rationale for the study

Toxoplasmosis is one of the most common zoonotic infections that is prevalent worldwide, caused by the protozoa parasite *Toxoplasma Gondi* (Dubey and Beatie, 1988). The infection not only affects animal's health, causing production and economic loss, but also continues to be a global human health risk (Tenter et al., 2000).

The sero-prevelance varies widely in different regions in the world, but is particularly prevalent in Europe, South America, and Africa (Petersen 2007). The prevalence is influenced by geography, environmental conditions, as well as cultural habits and the hygiene of people (Dubey and Beatie, 1988). The infection can be acquired by accidental ingestion of contaminated food or water with infective oocycts seeded by cats, by tissue cysts in raw or undercooked meat or transplacentally to the fetus during pregnancy (Dubey and Beatie, 1988).

Clinical symptoms of toxoplasmosis can vary. Eighty per cent of primary infections are asymptomatic and on the opposite, symptoms may be chronic making clinical diagnosis difficult (Studenicova et al., 2006). The clinical diagnosis must therefore be supported by a serological test.

Treatment includes antibiotics, folinic acid and corticosteroids. The infection can be particularly severe in those with immune deficits, and in babies who are congenitally infected. Prevention and education strategies are the key to avoiding future outbreaks (Dubey and Beatie, 1988).

Congenital toxoplasmosis is clearly related with certain neurological disorders such as seizures, blindness, mental retardation, hydrocephaly, cerebral palsy and nerve deafness (Roizen et al. 1995). The impact of acquired toxoplasmosis on neurological diseases is still not clear. Epidemiological surveys have reported that the prevalence of epilepsy in developing countries is higher than in industrialized countries (Senanayake and Roman, 1993), suggesting a role for infective aetiologies. Some studies have shown that a definitive infective aetiology of epilepsy can be determined in about 50% of all newly

diagnosed cases (Annegers et al., 1996). However, little is known about the potential etiological role of this protozoan in epilepsy.

A few studies have shown to be an association between latent acquired toxoplasmosis and the development of epilepsy (Yazar et al., 2003, Palmer, 2007.). Understanding the association between toxoplasmosis and epilepsy could be an important step in future efforts to reduce and prevent epilepsy and other learning difficulties in low resource epilepsy settings.

2.2. Purpose of the study

Probably the *Toxoplasma Gondii* infection has an association with epilepsy, either as a cause or a potential risk factor for its occurrence. There has been long-standing interest in investigating this possible association. However, the evidence for such a relationship is far from conclusive. The purpose of this study was to investigate the relationship between *Toxoplasma* seropostivity and cryptogenic epilepsy in children.

2.3. Libya as a case study

Libya is a developing country, where the environmental conditions and lack of awareness about toxoplasmosis' risks, are favourable for the transmission of this parasite. In addition, Libya has a National Epileptic Centre caring for many children with epilepsy of unknown origin and can provide a good resource for patients' enrolment in this study. This is also the first study to be conducted in Libya determining the prevalence of toxoplasmosis amongst both non-epileptic and epileptic children.

The data collected from this study will be useful to determine the association between epilepsy of unknown origin and the presence of anti-*Toxoplasma* antibody. Its potential role in pathogenesis of epilepsy will be assessed. Epidemiologic data can be used for the design of measures for the prevention of this disease.

2.4. Human Toxoplasmosis

2.4.1. Epidemiology

2.4.1.1. Global prevalence of toxoplasmosis

The infection has a worldwide distribution. One-third of the world's population has been exposed to this parasite (Dubey and Beatie, 1988)(Verma & Khanna 2013). The prevalence in humans varied significantly from region to region depending socioeconomic parameters, population habits and cultural factors (Boughattas et al. 2010). Epidemiological studies recording prevalence of *Toxoplasma gondii* infection around the world indicate considerable variation between countries (Figure 1). It is highly prevalent (> 60%) in South America and tropical region of Africa. Moderate prevalence (30 to 50%) have been found in countries of Central and Southern Europe and low prevalence (10 to 30%) have been observed in North America, South East Asia, Northern Europe and in Sahel African countries (Robert-Gangneux & Dardé 2012) (Pappas et al. 2009a).

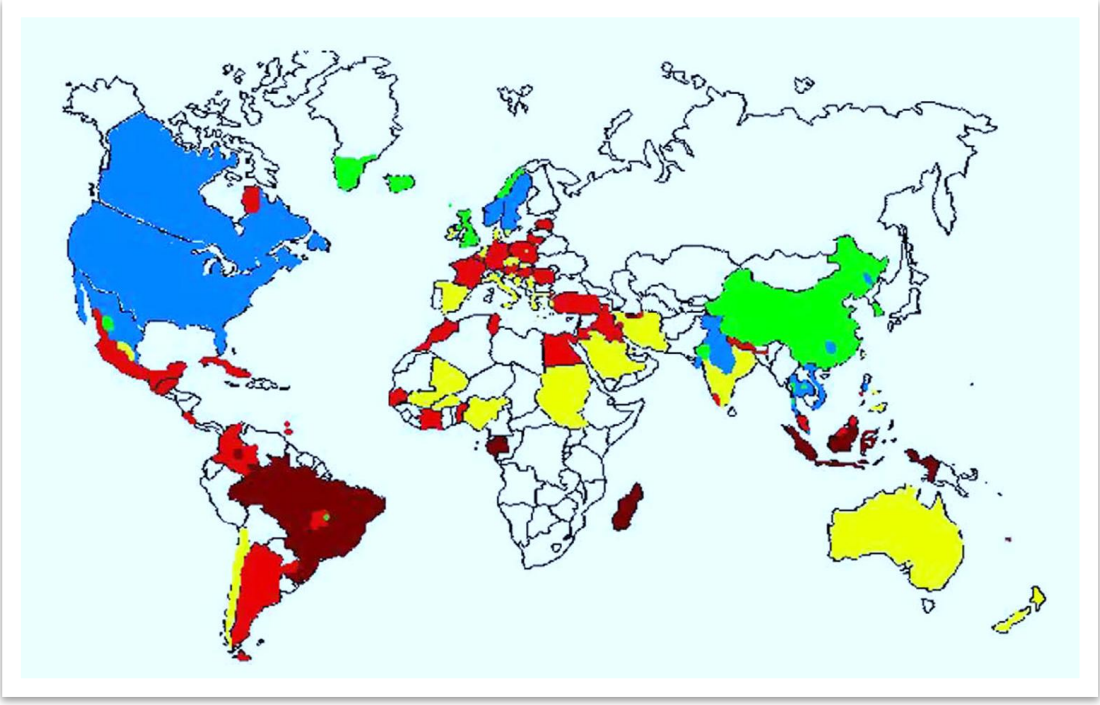
Some Serological research around the word showed that seroprevelance of *Toxoplasma* in several Latin-American countries including Brazil, Cuba, Jamaica, and Venezuela was even higher, with a range from 51–72% (Tenter et al., 2000). In Brazil, a higher seropositivity was found among the poor socio-economic groups. In different regions of Brazil, the seropositive rate varied between 37% and 91% (Rodrigues et al. 2015) Seroprevalence is high in some European countries, particularly France. It is up to 54% in southern European countries (Fromont et al. 2009) and decreases to 5–10% in northern, Sweden and Norway (Evengard et al., 2001). Prevalence in USA is 15.8% in the age group 12-49 year-old (Shin et al. 2009). However, over the past four decades age-specific prevalence has been decreasing in Europe (Welton, 2005). In France, serological surveys showed a decrease in *T. gondii* prevalence among pregnant women from 80% in 1960 to 44% in 2003 (Villena et al. 2010). The same result was seen in other countries. A national survey found a decrease in *T. gondii* prevalence in US-born persons aged 12 to 49 years, from 14.1% in 1988 to 9% in 2004 (Jones et al. 2007).

In most Asian countries the seroprevalence of *Toxoplasma gondii* infection is still low, 12.3% in China (Xiao et al. 2010),12.9% in Korea (Shin et al. 2009). But recent studies from Malaysia and India showed high seropositivity rates. Seroprevalence in Malays was 55.7 % (Nissapatorn et al., 2003), while it was 45% in India (Singh and Pandit, 2004). The seroprevalence in human immune-deficiency virus (HIV)-positive patients was 10.2% in Taiwan (Hung et al., 2005) and 39.3% in Iran (Daryani et al. 2014).

In African countries, numerous studies were performed in the early 1990s´. Seroprevalence was heterogeneous: Nigeria 20.8 %, Mali 21 %, Benin 3.6 %, Gabon 71.2 %, Madagascar 83.5 % and Senegal 40.2 % (Pappas et al. 2009a).

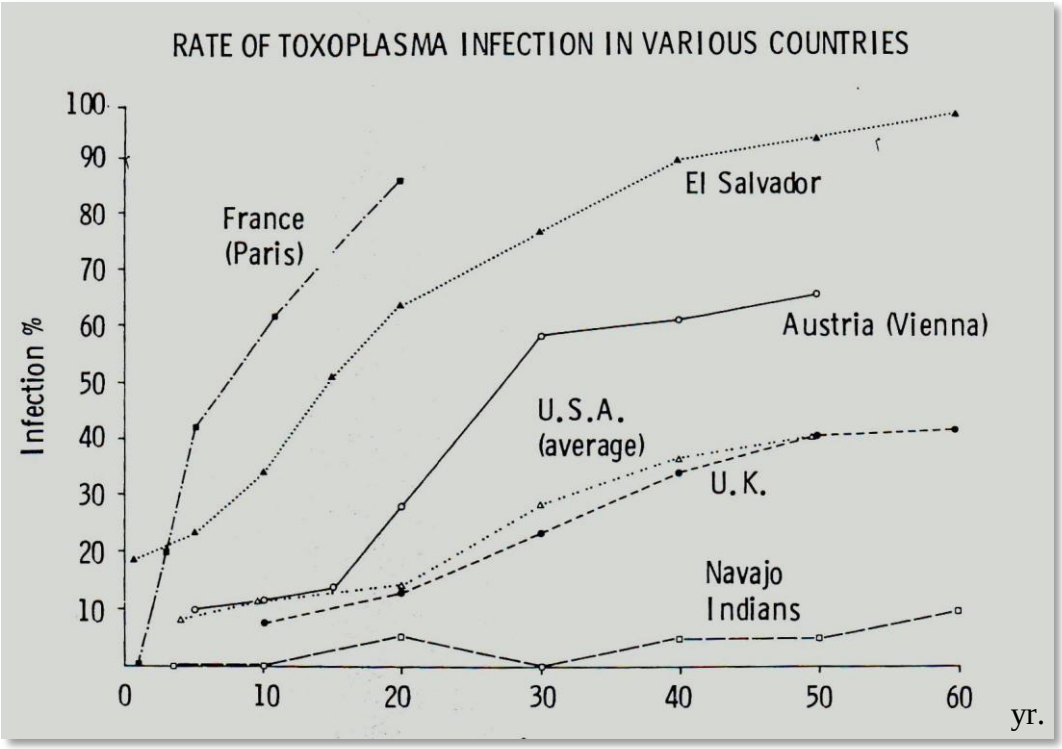
In Africa, toxoplasma seropositivity is considered to be a potential marker of HIV infection, due to the association between toxoplasma encephalitis and AIDS. African immigrants to the UK had a ten-fold higher incidence of symptomatic toxoplasma retino-choroiditis compared to white people born in Britain (Gilbert et al., 1999).

Figure 1: Prevalence of human toxoplassmosis.



Global status of Toxoplasma gondii seroprevalence. Dark red equals prevalence above 60%, light red equals 40–60%, yellow 20–40%, blue 10–20% and green equals prevalence <10%. White equals absence of data (Pappas et al. 2009b).

Figure 2: Increasing Toxoplasma seropositivity with age.



Rate of Toxoplasma gondii infection in various countries, showing increase of the Toxoplasma seropositivity with ages (Dubey and Beatie, 1988).

2.4.1.2. Global prevalence of toxoplasmosis in children

Information related to *T. gondii* infection among schoolchildren remains largely unknown to date. Due to the potentially serious implications of *T. gondii* infection on the foetus the majority of surveys focuses on childbearing age women, immunocompetent adults, and HIV/AIDS patients. The seroprevalence in Iran, Indonesia, Slovak, and Brazil was high, ranged from 20.9% to 68.4% (Souza et al. 1987)(Fan et al. 2012). In developed countries, the prevalence of *T. gondii* infection among primary schoolchildren (PSC) was low, with a range of 0.0% to 11.0% in Japan and in Ireland (Fan et al. 2012) (Taylor et al. 1997). Taylor et al. found seroprevalence of 12.8% in Irish children aged 4-18 years, with no difference between the sexes. Prevalence increased with age and was higher in countryside than towns, 16.6% and 10.2% respectively (Taylor et al. 1997).

In Africa, the majority of surveys have been carried out on adults, women of reproductive age and on HIV/AIDS patients (Hammond-Aryee K, Esser M 2014). However, studies concerning the seroprevalence of *T. gondii* in children are limited; few studies have been performed in some countries. The seroprevalence of *T. gondii* infection among primary school children was 24% in Nigeria (Gyang et al. 2015), 37.5% in Somalia (Ahmed et al. 1988) and 52.1% in Madagascar (Dromigny et al. 1996). The overall seroprevalence of *T. gondii* infection ranged from 21.49% to 63.1% in Democratic Republic of São Tomé and Príncipe (DRSTP) and West Africa (Fan et al. 2012)(Fan et al. 2006), while in the Republic of the Marshall Islands it was found to be 54.8% (Fu et al. 2014).

In Asian countries prevalence was lower. *T. gondii* infection among children aged 6-11 years was 16.0-15.3% (Xin et al. 2015)(Meng et al. 2014). In Iran was 10% (Ali et al. 2007). In Indonesia prevalence at 0-9 years in both genders was less than 10%, but in those at ages over 10 years there were more than 50%, suggesting an extremely high transmission rate (Konishi et al. 2000). In Bahrain, seroprevelance of toxoplasmosis was 9.3% (Tabbara & Saleh 2005) .

In Latin American, a high seroprevalence of toxoplasmosis (55%) was found in children aged 13–17 yr. in Chile (Munoz-Zanzi et al. 2016). In Panama, seroprevalence has been reported to be 13% by age of 6 years (Frenkel et al. 1995). In Brazil, prevalence rates ranged from 17.5% (Dattoli et al. 2011) to 46.4% (Lopes et al. 2008). Seroprevalence according to age was: 2 to 9 years: 40.0%; 10-19 yr.: 60.4% (Rey & Ramalho 1999).

In 1999, the prevalence of *Toxoplasma gondii* infection in Guatemalan children aged from 2 months to 2 years was 12.4%. In 2003, antibody prevalence increased to 43% at the age of five years (Jones et al. 2005).

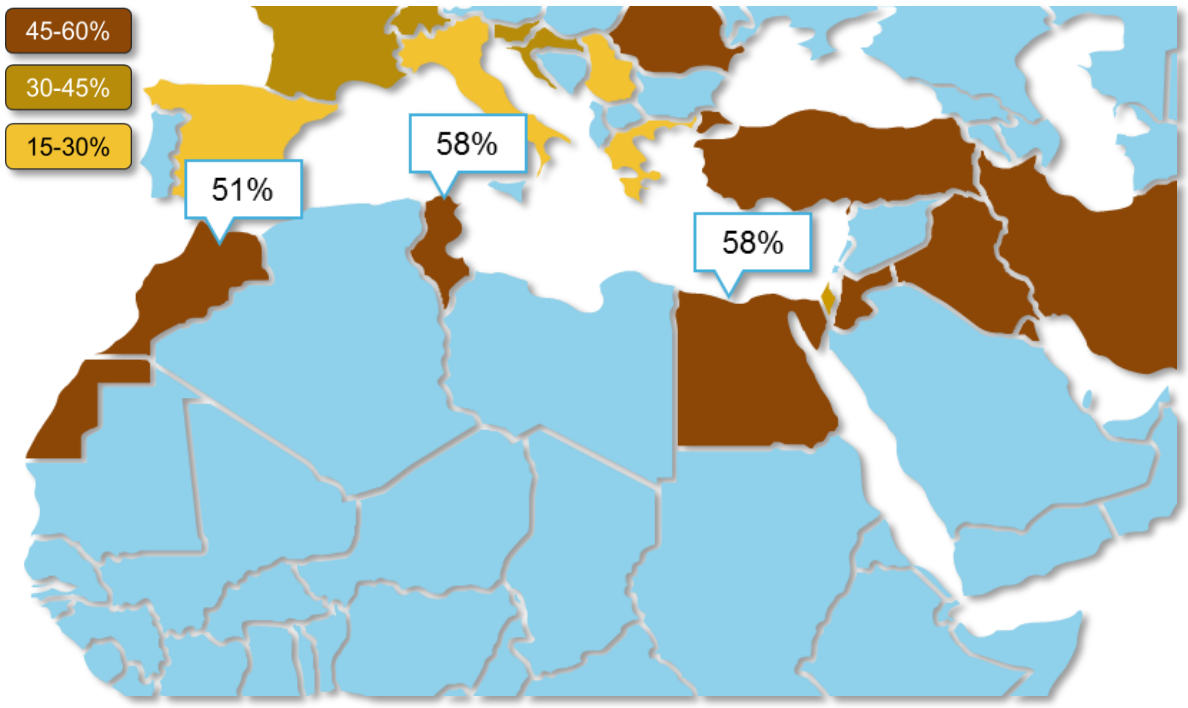
The discrepancy in seroprevalence in different countries could be attributable to different ethnicity, traditional culture, as well as food habits (Jones et al. 2009)(Cenci-Goga et al. 2011).

2.4.1.3. Prevalence of toxoplasmosis in North Africa and Middle East

In North Africa, there is an average seroprevalence rate of 58%, with a progressive rise from 24% at 10 years of age to 70% at 30 years of age (Bouratbine et al. 2001). Recent

review by Pappas et al. summarized prevalence rates in neighbouring countries Egypt (57.9 %) and Tunisia (58.4 %) (Pappas et al. 2009a). Whilst in Morocco is 50.6 % (Hussein et al. 2001).

Figure 3: Prevalence of toxoplasmosis in South Europe, North Africa and Middle East.



Data from Pappas et al. 2009.

2.4.1.4. Prevalence of toxoplasmosis in Libya

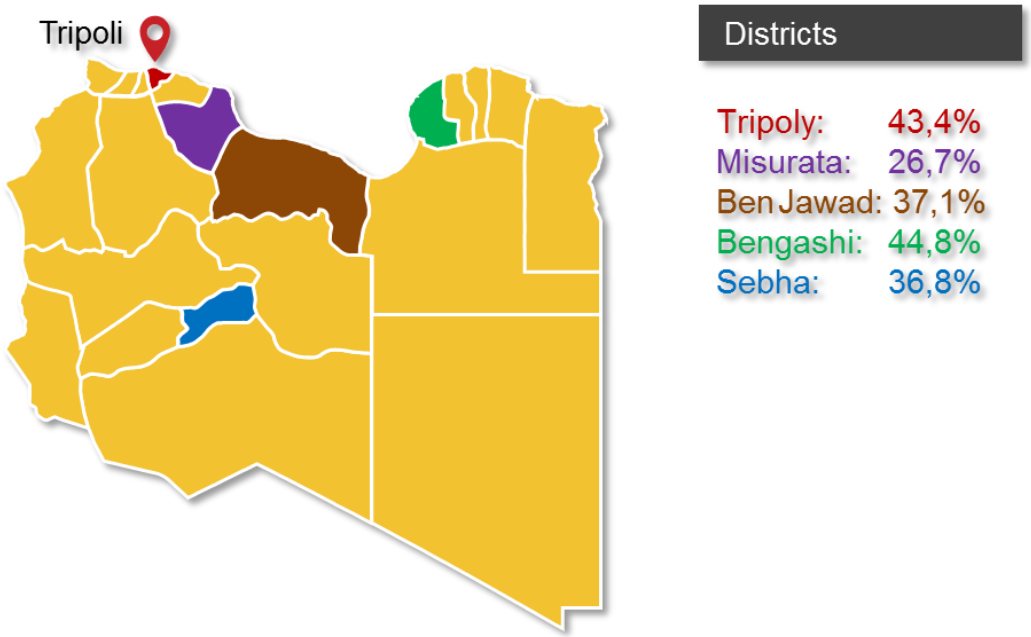
In spite of the fact that toxoplasmosis is one of the most diseases distributed worldwide. Very little amount of literature has been published on seroprevalence and the epidemiology of the disease in the Libya. No epidemiological analysis was done at national level.

Some studies have been conducted to determine the prevalence of *T. gondii* among pregnant women, carried out in some main cities such as Tripoli and Benghazi. However, like most developing countries, there is no national programme for screening pregnant women for toxoplasmosis in Libya. The first report in Benghazi was done by

Legnain and Prawecka (1983), who found seropositivity in 45.8% of pregnant women (Legnain M. M. and Prawecka M 1983). Seroprevalence of toxoplasmosis among pregnant women in Benghazi oscillated from 44.8 % to 50% (Mousa et al. 2011) (Kassem & Morsy 1991) (Magrhi S et al. 2003). In the area of Tripoli, seroprevalence was 43,4% in adult females (Khadre & El Nageh 1987). In Misurata, 26,7% were positive in first trimester of pregnancy (Ramadan Sariti et al. 2015). In the Sebha region prevalence in pregnant women was 36,8% (El-Sayed & Almannoni 2016). The overall incidence rates of *T. gondii* among pregnant and non-pregnant women from Ben Jawad were 37.16% and 35.5% respectively (Boshapor & Kassem 2015). Setta found low prevalence (18,6%) in non-pregnant women in Tripoli in 2006-2007 (Setta AM 2008).Prevalence in women with spontaneous abortion was 45% in Tripoli in 2002-2007 (Gashout et al. 2008), and decreased to 38,5% in 2010 (Gashout et al. 2016).

Sero-prevalence of toxoplasmosis among Libyan psychiatric patients in Tripoli was 61.7%. It was higher than in non-psychiatric controls, suggesting some pathogenic relation between toxoplasmosis and psychiatric diseases (Elsaid MM, Dia Eddin EE 2014). The main reasons for this high prevalence rate of toxoplasmosis in Libya is yet unclear as no adequate studies regarding transmission modes were available.

Figure 4: Prevalence of toxoplasmosis in women and pregnant women in Libya.



Information concerning *T. gondii* infection among schoolchildren is scant. Only one old published study investigated about the prevalence of *Toxoplasma gondii* infection in schoolchildren. In the area of Tripoli, seroprevalence was 43.7% in 1980 schoolchildren of 7–18 years of age (Khadre & El Nageh 1987).

2.4.2. Transmission

The only known definitive hosts for *Toxoplasma gondii* are members of family *Felidae* (domestic cats and their relatives). Intermediate hosts in nature (including birds and rodents) become infected after ingesting soil, water or plant material contaminated with *Toxoplasma gondii oocysts* (Jones et al. 2003)

2.4.2.1. Pathways for *Toxoplasma gondii* infection transmission to humans

Epidemiological surveys are the most useful way for assessing the relative importance of different sources of *T. gondii* infection in humans (Zuber and Jacquier, 1995). Consumption of raw meat (undercooked meat), drinking unboiled milk and and untreated water, contact while cleaning cat litter and soil contact has been shown to be an important risk factor in several studies (Sousa et al., 1988). Transplacental transmission from mother to foetus by *tachyzoites,* can cause severe neurological and ophthalmological sequels (Carruthers & Suzuki 2007).

The importance of these modes of transmission may vary in different populations. *Toxoplasma gondii* has been identified in saliva, sputum, within mucosa of bladder and intestine and in the kidneys of infected humans. Contaminated human urine and faeces may be theoretically a source of infection, but transmission by this source has never been proved (Remington and Desmonts, 1983). Less commonly, toxoplasmosis has been transmitted by transplantation of an infected organ or thorough contaminated blood transfusion (Hill & Dubey 2002). Laboratory workers who handle infected animals or needles are at great risk. A total of 47 laboratory-acquired cases have been reported, 81% of them were symptomatic (Herwaldt, 2001). Transmission by sexual activity including kissing is probably rare and epidemiologically unimportant (Zuber & Jacquier 1995).

2.4.3. Biology of Toxoplasma gondii

Toxoplasma. gondii is an obligate intracellular parasite. It has a complex life cycle consisting of sexual and asexual cycles (Figure 5) and different stages (Dubey and Beatie, 1988) (Robert-Gangneux & Dardé 2012) (Tenter et al. 2000). The sexual cycle takes place only in primary host family *Felidae*, including domestic and feral cats. The asexual cycle takes place in a wide variety of intermediate hosts, including humans.

2.4.3.1. Sexual cycle

It's an entero-epithelial cycle. It takes about 3-10 days and occurs only in the gut of the definitive hosts felids.

Oocysts are eliminated by cat feces. *Oocysts* undergo sporulation in the soil, developing infective *sporozoites*. Each *oocyst* develops two oval *sporocyst*. Four *sporozoites* differentiate and grow inside each *sporocyst*. *Oocyst* become infective within 5 days depending on environmental conditions (Dubey and Beatie, 1988).

Figure 5: Life cycle of Toxoplasma gondii.

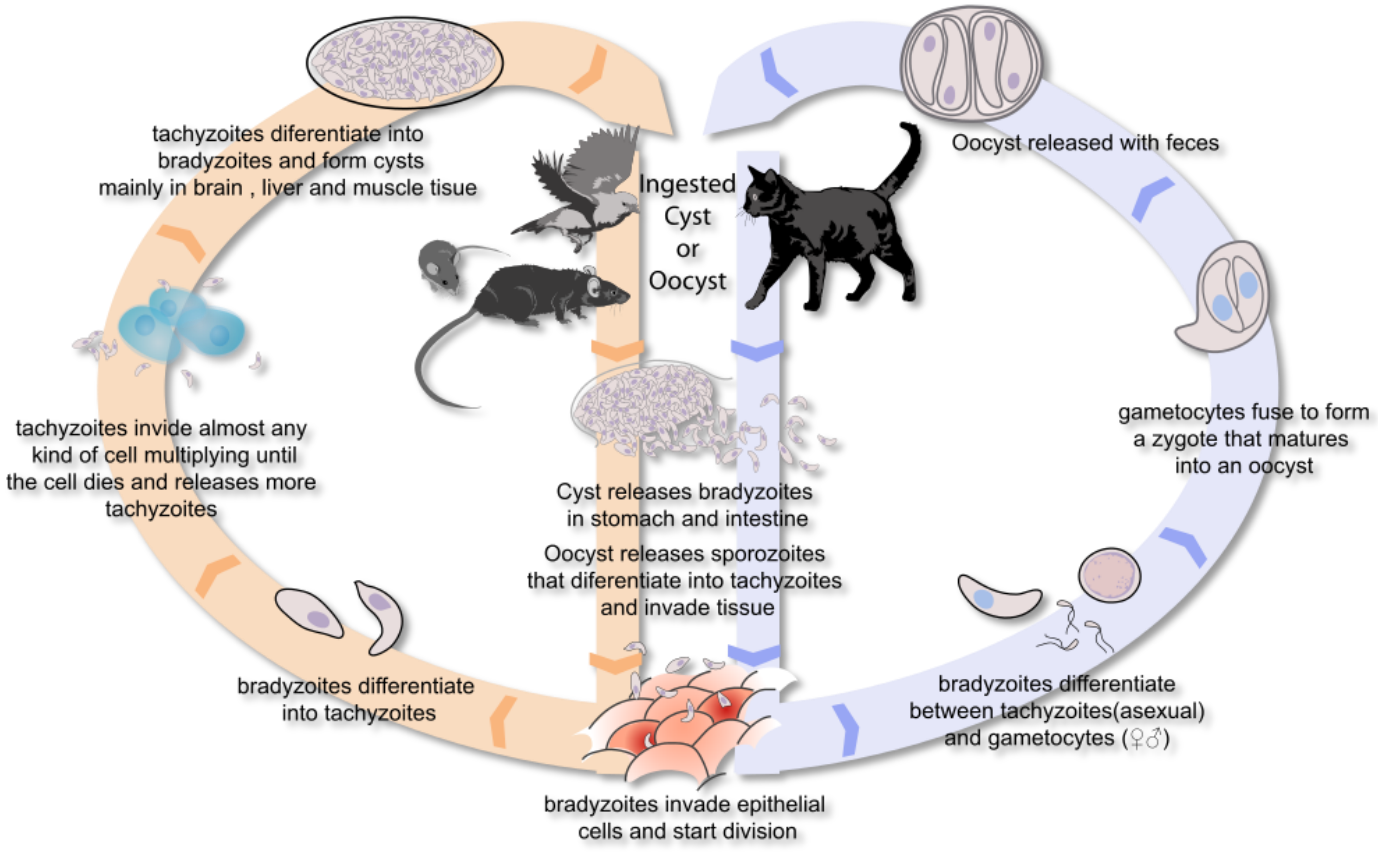


Figure 6: Oocysts (Dubey et al, 1998).

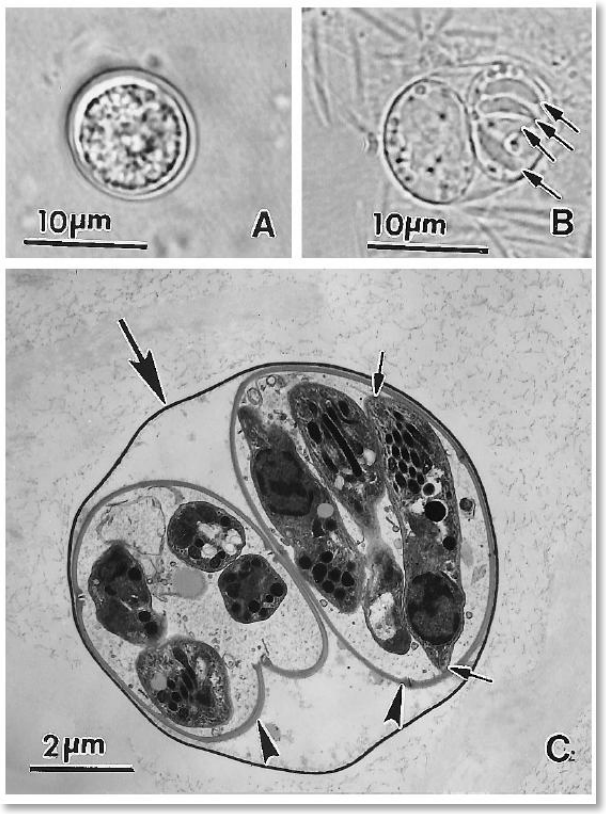


Image from Dubey , Lindsay and Speer, Clin Microb Reviews, 1998.

A. Unsporulated oocyst.
B. Sporulated oocyst showing two sporocysts. Right sporocyst shows four spozoites developing inside (arrows).
C. Sporulated oocyst with four sporozoites inside each sporocyst.

When cats ingest *oocysts* shed from other cats, *sporozoites* are liberated in gut lumen. *Sporozoites* where they differentiate into their rapidly multiplying form (tachyzoite). *Tachyzoites* invade epithelial cells and inside the enterocyte, the parasite undergoes several rounds of division. Intracellular divisions generate new tachyzoites and micro-(male) and macro-(female) gametocytes. Fertilized gametocytes form a zygote or oocyst that is shed into the environment with the cat faeces.

2.4.3.2. Asexual cycle

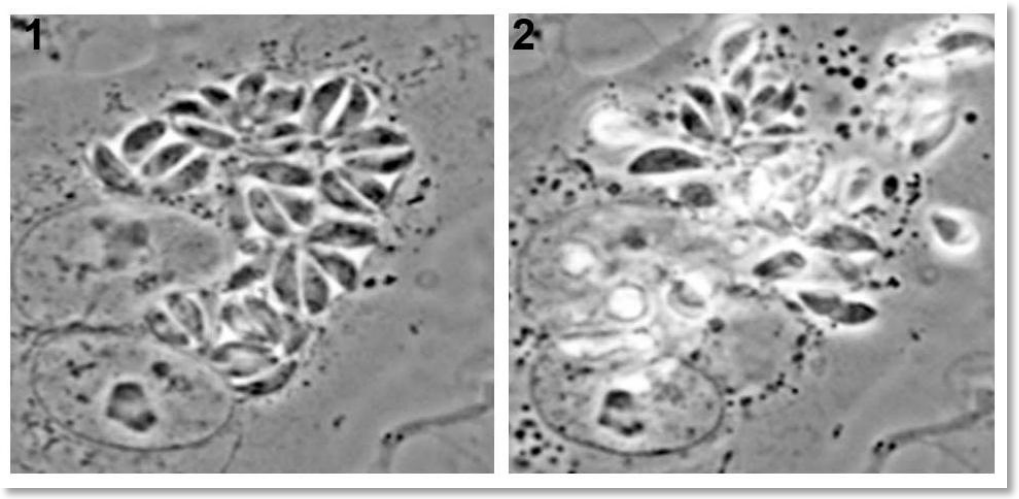
Following ingestion of the oocyst by intermediate hosts (human and animals), extra-intestinal cycle of infection develops. The *sporozoite* differentiates into the rapidly multiplying form *tachyzoite. Tachyzoites* are distributed via the blood stream thus establishing the acute infection parasitemia (acute stage). These *tachyzoites* invade neural and muscle cells and other tissues. Inside the cells *tachyzoites* are surrounded by a membrane (parasitophorous vacuole) and multiply as rosettes. Finally, the cell wall is

broken and new *tachyzoites* are liberated. Some *tachyzoites* transform into a slowly dividing form known as *bradyzoites* and begin to multiply forming *tissue cysts* (chronic stage).

Latent *bradyzoite tissue cysts* are the terminal life-cycle stage in the intermediate host. They may persist for the life of the host or ruptured and released bradyzoites become active tachyzoites and start to proliferate again (Dubey and Beatie, 1988, Remington and Desmonts, 1983).

Meat and other tissues of infected animals include *tissue cysts*. After ingestion of *tissue cysts*, *bradyzoites* are liberated in the digestive tract of definitive or intermediate hosts, transform into *tachyzoites* and begin tissue invasion.

Figure 7: Intracellular tachyzoites.



Sarah Myers, 2014. 1: HeLa cell filled by tachyzoites. 2: tachyzoites actively emerge through cell wall.

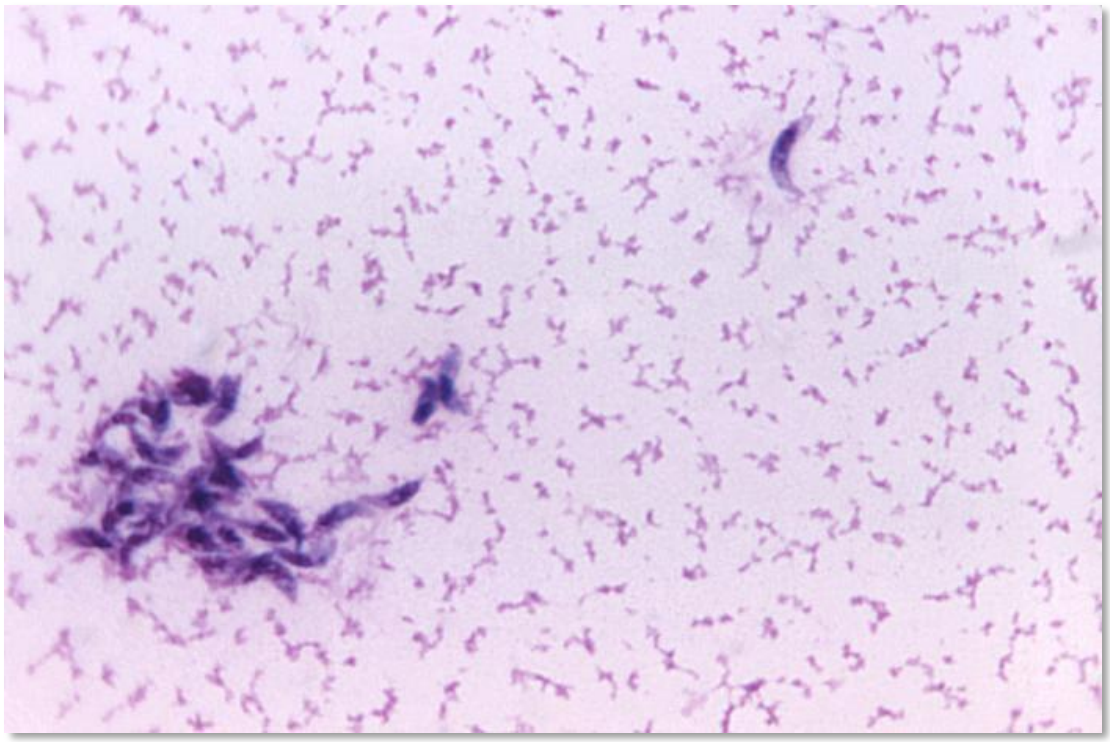
2.4.3.3. Toxoplasma morphology

***Tachyzoites**:* asexual proliferative forms involved in cell invasion. It has a semilunar shape, measures 3 to 7 microns (Figures 7 and 8). They are obligate intracellular microorganisms, but they can survive outside the cells in various body fluids for periods of hours or days. They convert into bradyzoites inside cells (Montoya and Liesenfeld, 2004, Dubey and Beatie, 1988). They do not survive digestive activity of the stomach and are not infectious by ingestion. However, they can cause trans-placental infection.

Tissue cysts: tissue cysts size ranges from 10-200 microns in diameter. Found in the brain,skeletal and cardiac muscles. They are rounded in brain and elliptic in muscle cells (Montoya and Liesenfeld, 2004). Inside they include *bradyzoites*, similar to *tachyzoites* but smaller and divide more slowly. They are more resistant to digestive enzymes and, just as the *oocysts* are infectious for the animals that ingest them. They survive in normal cooling temperatures, but can be destroyed by freezing, thawing and usual cooking temperatures.

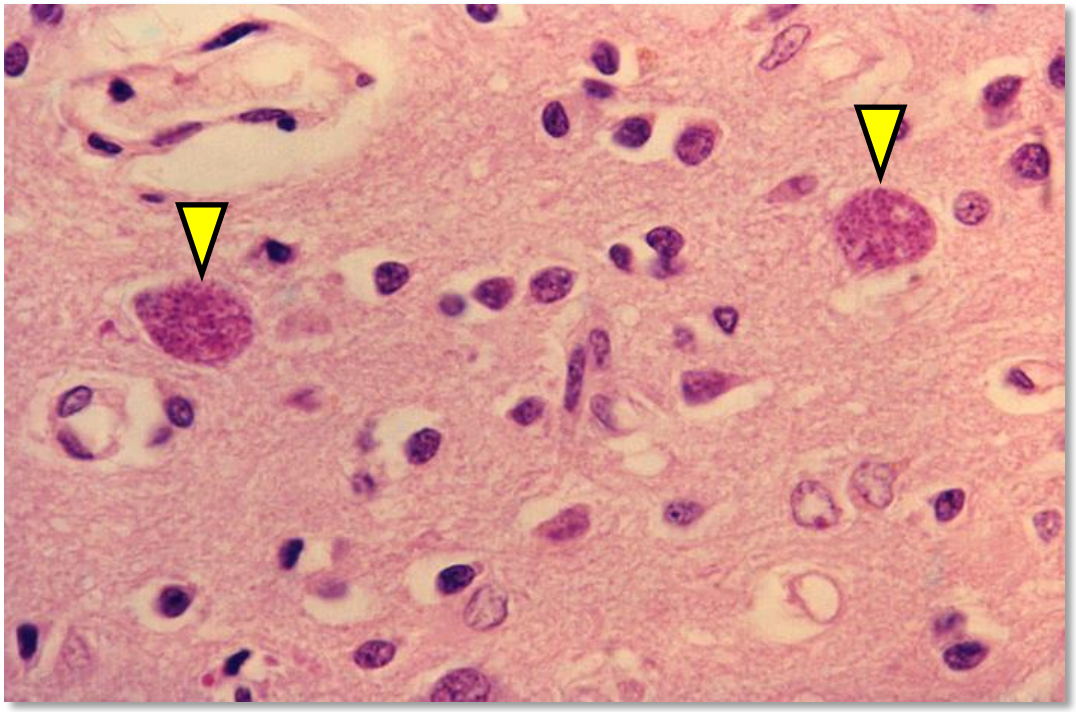
Oocysts: they have ovoid shape, measure 10 to 12 microns in diameter (Figure 6). They possess a thick wall that makes them resistant to most environmental factors. They can be destroyed at cooking temperatures. They are their immature form, the center of the cyst appears with two *sporocysts* and later four *sporozoites* can be identified in each *sporocyst*. This form is the one that participates in the dissemination of the feline parasite to another animal and fecal-oral route. The number of *oocysts* eliminated in the fecal matter can reach 10 million daily for periods of 20 days.

Figure 8: Tachyzoites.



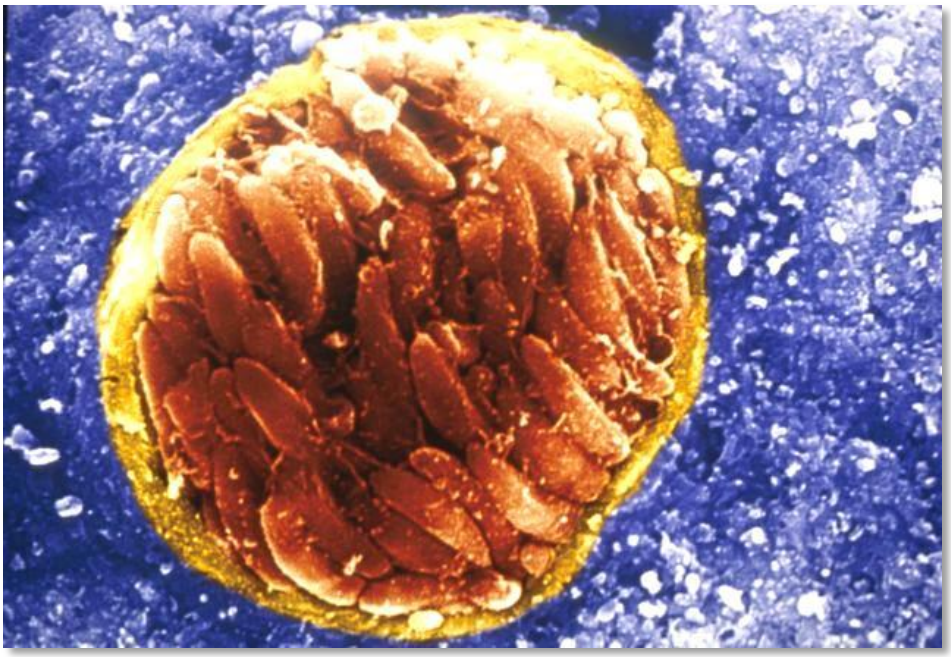
Toxoplasma gondii tachizoites in mouse peritoneal fluid. CDC.

Figure 9: Brain tissue cysts of Toxoplasma gondii.



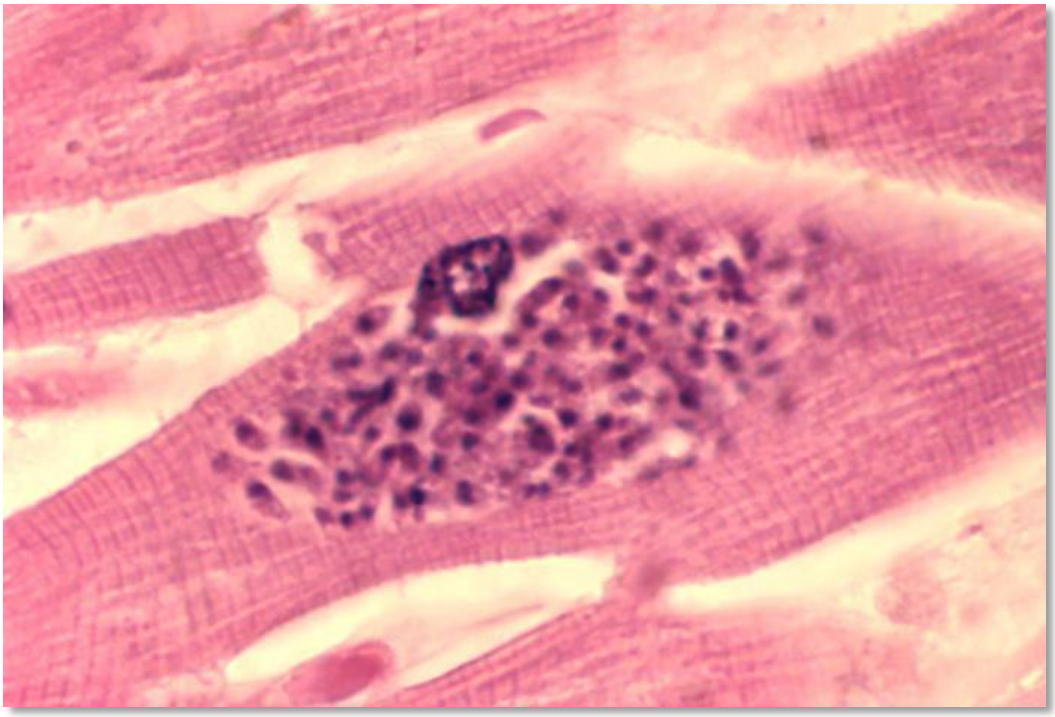
Brain tissue cysts. JWM. Gold.. 1986. Public Healh Image Library (PHIL)-CDC.

Figure 10: Bradizoites.



Bradizoites inside tissue cyst in brain of infected mouse. David Ferguson. EurekaAlert.org.

Figure 11: Tissue cyst in myocardial muscle.



Histopathology of active toxoplasmosis of myocardium. EP. Ewing, 1984. PHIL-CDC.

2.5. Diagnostic of toxoplasmosis

Diagnosis of toxoplasmosis is made indirectly with serological methods and directly by detection of the parasite or its DNA (Montoya & Liesenfeld 2004).

2.5.1. Serology

***IgG antibodies*:** The most commonly used tests, usually appear within 1–2 weeks, peak within 1–2 months than then decline and usually persist for life. It measurement of IgG antibody are the DT, the ELISA, the IFA, and the modified direct agglutination test (Montoya 2002). IgG titers over 8 IU/mL are generally accepted as positive, but there are differences between the results obtained by different manufacturers of tests (Villard et al. 2016). High titers are related to recent infection, symptomatic infection (Vidal et al. 2011) and reinfection or reactivation (Dzitko et al. 2006). Sabin-Feldman dye test and inmunoblot techniques (Sroka et al. 2016) are the reference methods.

Avidity of IgG antibodies: It is the better test for confirm recent infection. Method described by Hedman et al. is based on the distinct strength of the binding between

antigen and antibody in infection acute and chronic (Hedman et al. 1989). IgG with low avidity predominates in the early stages, whereas in chronic infection it occurs the opposite situation. In fact, there are IgGs of high and low avidity always; what varies is the relative proportion each type. The presence of highly avid IgG antibodies in excess of 30% excludes acute infection.

***IgM antibodies*:** An IgM test is still used to determine recent infection. IgM antibodies remains positive for over 2 years (del Castillo 2005). Recent infection is better diagnosed by detecting low avidity IgG. Confirmatory testing should always be performed. Commonly measured by double-sandwich or capture IgM-ELISA kits, the IFA test and the immunosorbent agglutination assay (Montoya 2002).

***IgA and IgE antibodies*:** Are detected in sera of acutely infected adults and congenitally infected infants by use of ELISA or ISAGA (Montoya 2002).

2.5.2. Polymerase Chain Reaction

It has successfully been used to diagnose congenital, ocular, and cerebral and disseminated toxoplasmosis by detect *T. gondii* DNA in body fluids and tissues (Montoya 2002). At the moment, the PCR techniques allow to avoid the culture in the majority of the situations. In the CSF sensitivities are obtained between 40 and 50%.

2.5.3. Isolation of T. Gondii

The diagnosis of certainty can be established by cell culture. The culture techniques in the experimental animal should be limited to situations of special diagnostic difficulty. Intraperitoneal inoculation to mouse is the reference method.

2.5.4. Histological diagnosis

Infection is confirmed by demostration of *tachyzoites or tissue cysts* in tissue sections or smear of body fluid (e.g., CSF amniotic fluid and blood in patients with AIDS). Direct immunofluorescence (Matossian et al. 1977) and immunohistochemical techniques are the preferred methods for direct identification of *Toplasma gondii* in tissue samples.

2.6. Toxoplasmosis and neurological outcomes

Infection with the toxoplasma parasite can cause several neurologic syndromes:

2.6.1. Congenital toxoplasmosis and CNS

Women infected with *Toxoplasma* before conception, with rare exceptions, do not transmit the infection to their foetuses. Congenital infection occurs when a woman is infected acutely or has a reactivation during pregnancy (Jones et al., 2003). It is not transmitted in subsequent pregnancies. In Europe it estimated to occur in between 1-10/10000 live births (Antoniou et al., 2004). The incidence and severity of congenital toxoplasmosis depends on the gestational age at which maternal infection occurs. It is more frequent as the pregnancy progresses, ranging from less than 2% at gestational week 4 to more than 80% at 36 weeks gestation (Montoya and Liesenfeld, 2004). However, the consequences become less severe as the gestation progresses. An estimated one half of untreated maternal infections are transmitted to the foetus. (Dunn et al. 1999). If untreated, congenital toxoplasmosis can be associated with severe and even fatal disease (Jones et al., 2003; Paul et al., 2001).

2.6.1.1. Asymptomatic congenital toxoplasmosis

Most infants infected in utero are born with no obvious signs of toxoplasmosis on routine examination, includes babies with positive serology that persists longer than 12 months. They may develop learning and visual disabilities later in life.

2.6.1.2. Symptomatic congenital toxoplasmosis

A small minority of babies will have symptomatic congenital toxoplasmosis. The classical triad of signs suggestive of congenital toxoplasmosis include chorioretinitis, intracranial calcifications (Figure 12) and hydrocephalus. Other clinical manifestations also occur, such as hepatosplenomegaly, pneumonia, thrombocytopenia, lymphadenopathy or myocarditis (Table 1).

Diagnosis can be confirmed serologically by IgM positive, with an IgG titre significantly greater than mothers (Holliman, 2003) or by immunoblotting of IgG antibodies (Chumpitazi et al. 1995). *Toxoplasma gondii* DNA can be detected in

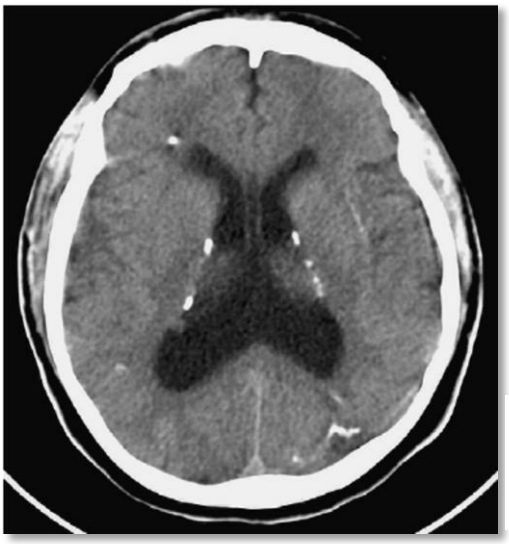
amniotic fluid, placenta, blood, CSF or urine using molecular methods such as PCR (Meenken et al., 1995; Zhang et al., 2002).

Table 1: Clinical manifestations of congenital toxoplasmosis.

Signs and symptoms of congenital toxoplasmosis in infants and children*	
Intracranial calcifications†	Hepatomegaly
Hydrocephalus†	Splenomegaly
Chorioretinitis†	Anaemia
Abnormal spinal fluid	Thrombocytopenia
Microcephaly	Jaundice
Spasticity and palsies	Lymphadenopathy
Convulsions	Maculopapular rash
Visual impairment	Fever
Deafness	Growth retardation
Learning disabilities	
Mental retardation	

***Most neonates with congenital toxoplasmosis are asymptomatic on routine new-born examination. †Signs of the classic triad suggesting the presence of congenital toxoplasmosis (Jones et al., 2003).*

Figure 12: Hydrocephalus and brain calcifications in congenital toxoplasmosis.



Dr. Ahmed Abdel Khalek

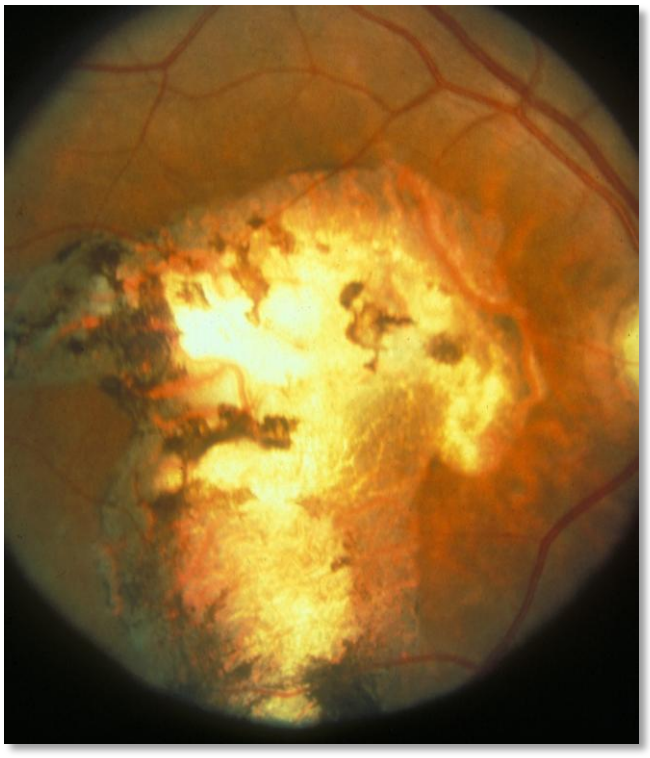
2011.

2.6.2. Retinal toxoplasmosis

Progressive and recurrent focal necrotising chorioretinintis is the hallmark of congenital infection or infection after birth (Figure12). In congenital infection, patients are often asymptomatic at birth. Clinical symptoms are most commonly manifested during infancy and adolescence (Roizen et al., 2006). It is seen in 75-80% of cases and 85% are bilateral (Wu, 2007). It was reported to be a rare feature of acquired infection, with only 1-3% of patients in old case-series (Gilbert and Stanford, 2000), but recent studies shows increased importance of acquired infection as aetiology of retinal toxoplasmosis (Arantes et al. 2015) (Balasundaram et al. 2010).

Cysts deposited on the retina cause focal necrotizing retinitis; those deposited in the optic nerve cause optic neuritis or papules called Jensen disease (Wu, 2007). Further abnormal ocular features include optic nerve atrophy, strabismus, microophthalmus, cataracts and iris abnormalities (Meenken et al., 1995).

Figure 13: Retinal toxoplasmosis.

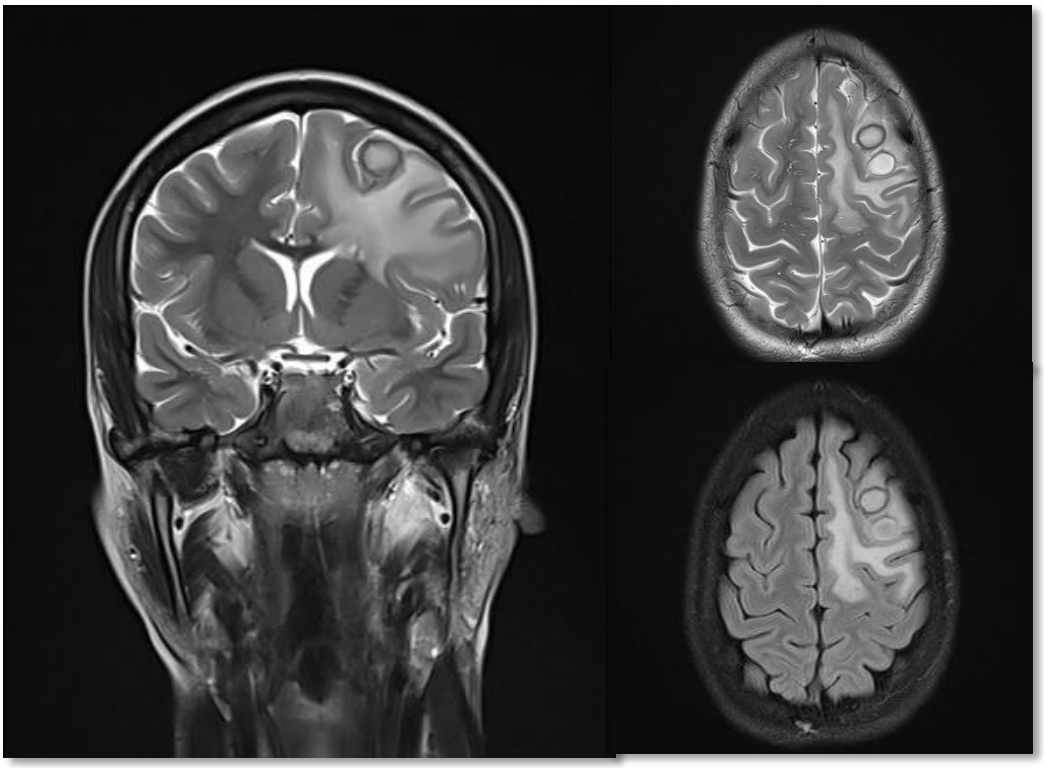


Central retina scar from toxoplasmosis chorioretinitis.

2.6.3. Brain toxoplasmosis in inmmunodefiency

In immunocompromised patients the central nervous system (CNS) is the most commonly affected site and approximately 2.5% of AIDS patients have been diagnosed with cerebral toxoplasmosis. Immunosuppression may be caused by AIDS or therapies for malignancies, transplants, or lymphoproliferative disorders. Toxoplasmosis can be due to either a newly acquired infection or as result of reactivation of latent *Toxoplasmo*sis (Montoya and Liesenfeld, 2004). Reactivation most often involves the central nervous system, and symptoms can include meningoencephalitis or symptoms of a mass lesion. CNS toxoplasmosis commonly presents with; focal neurological signs, motor weakness and speech disturbances (Montoya and Liesenfeld, 2004). Chorioretinitis and pneumonitis are more frequent in recipients of bone-marrow transplants. Moreover, multi-organ failure and septic shock may occur (Montoya and Liesenfeld, 2004). Skin involvement is rare (Leyva and Santa Cruz, 1986).

Figure 14: Brain toxoplasmosis in HIV infected girl with severe inmmunodeficiency.



2.6.4. Brain toxoplasmosis in immunocompetent host

The severity of *Toxoplasma* infections is correlated with the immune status of the infected subject. Toxoplasmosis in immunocompetent adolescents or adults is generally an asymptomatic infection in 70% to 90% of cases.

About 10 to 20% of patients present mild symptoms, as painless lymphadenopathy, fever, fatigue and malaise. All of which usually resolve within one week to three months without specific treatment (O'Connell et al., 1993). In rare cases ocular infection with visual loss can occur (Ryning and Mills, 1979).

A generalized transient non-pruritic maculopapular rash may occur, most prominent on the trunk and proximal extremities. Rash may be accompanied by fever, malaise, myalgia and sore throat (Ryning and Mills, 1979; O'Connell et al., 1993). Other clinical findings are very infrequent. Pericarditis, pneumonitis, nephritis, hepatitis, polyneuritis, haemolytic anaemia are described (Sano et al., 2000)(Cook et al 2003).

Encephalitis occurs exceptionally in immunocompetent humans (Lescop et al. 1995; Hoti et al. 2016; Akturk et al. 2017). Initial neurological symptoms are equivalent to those observed in HIV patients, headache, vomits, focal seizures, psychiatric symptoms and somnolence (Barbic 2014; Alapatt et al. 2009; Li et al. 2014). Post infectious encephalomyelitis was also described after acute *Toxoplasma gondii* infection (Aksoy et al. 2013).

2.7. Epilepsy

2.7.1. Introduction

Epilepsy is one of the world's most prevalent non-communicable diseases that affect nearly 50 million patients worldwide (Ngoungou et al. 2015). It is one of the most common serious neurologic diseases, associated with social stigma and significant economic costs (Benamer & Grosset 2009).

The prevalence of epilepsy is relatively high in many different parts of the world, between 2.3-44/1000 in all age groups. Among the paediatric age group, the prevalence of epilepsy is 18.5 (0.8-49) /1000 (Aydin et al. 2002).

The epilepsies are an important cause of neurological morbidity in children. Factors that are known to increase risk of the epilepsies in children account for only 25% to 45% of cases. Aetiologies include congenital malformations of the central nervous system (CNS), CNS infections, head trauma, some inherited metabolic diseases and genetic factors (Cowan 2002).

The International League Against Epilepsy (ILAE) defined Epilepsy as a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures and by neurobiological, cognitive, psychological and social consequences (Fisher et al. 2005).

It is classified into idiopathic for which there is no apparent cause, cryptogenic if an aetiology is unknown, but an underlying brain disease is suspected (Yazar et al. 2003) and secondary epilepsy if there is a known cause such as congenital abnormalities, trauma or hypoxia (Akyol et al. 2007). Cryptogenic epilepsies comprises about 20% of all epilepsy syndromes (Mrcp et al. 1992)

Although the rates of idiopathic ⁄ cryptogenic epilepsy (60–70%) and symptomatic epilepsy (30–40%) are similar in developing and developed countries, the causes of symptomatic epilepsy are different. In the developing countries infections of the central nervous system, whether acute or chronic–recurrent, are the most important cause of seizures and acquired epilepsy (Singhi 2011). Cryptogenic epilepsies comprise about 20% of all epileptic syndromes.

2.7.2. Epidemiology

2.7.2.1. Global prevalence of epilepsy

The World Health Organization estimates that epilepsy affecting over 50 million people, of whom 80% live in poor countries (http://www.who.int/healthinfo/statistics) (WHO 2001) (Wagner & Newton 2009). In 75% of cases, epilepsy begins in childhood (Pal et al. 2000) (Jallon 1997) (Senanayake & Roman 1993). The average annual rate of new cases (incidence) of epilepsy is approximately 5-7 cases per 10,000 children, from birth to age 15 years (Cowan 2002). Its burden is higher in developing countries than industrialized countries, particularly in Africa and Latin America (Nicoletti et al. 2002). In Africa, the median prevalence is 5-74 ⁄ 1000 (Preux & Druet-Cabanac 2005), in South America the range is 6-43 ⁄ 1000 (Burneo et al. 2005), and in Asia it is 2-14 ⁄ 1000 (Mac et al. 2007) compared to 3-8 ⁄ 1000 in Europe (Forsgren et al. 2005) (Montano et al. 2005). The great variability of prevalence rates is a reflection of different socioeconomic and sanitary situations and of individual study designs and methodologies (Eriksson & Koivikko 1997).

2.7.2.2. The epidemiology of the epilepsies in children

Epilepsies are an important cause of neurological morbidity in children. The incidence of epilepsy is approximately 5-7 cases per 10,000 children from birth to age 15 years, and in any given year, about 5 of every 1,000 children will have epilepsy.

Some epileptic syndromes are unique to children, including infantile spasms, Lennox-Gastaut syndrome and absence seizures. Febrile seizures and neonatal seizures are relatively common types and markers of risk of later epilepsy.

The aetiology of most cases of the epilepsies remains obscure. However, there are some conditions that are known to increase risk of epilepsy in children. They include congenital malformations of the central nervous system, severe head trauma, CNS infections, certain inherited metabolic conditions and genetic factors. However, these account for only 25% to 45% of cases (Cowan 2002).

2.7.2.3. Prevalence of epilepsy in Arabic countries

Epidemiologic data are lacking from the majority of Arabic countries. The estimated median prevalence of epilepsy in Arab countries is 2.3/1,000 (varying from 0.9-6.5/1,000). This is just within the range found in Europe and Asia (Forsgren et al. 2005) (Mac et al. 2007). The prevalence rate is approximately 2-fold higher in children and young adults, compared to middle age and in male more than female. All the studies from the Arab states found generalized seizures to be more common than partial seizures; idiopathic epilepsy represents 73.5-82.6% of cases (Benamer & Grosset 2009).

2.7.2.4. Prevalence of epilepsy in Libya

Epidemiologic studies and general profile of epilepsy in Libya were lacking in particular data concerning aetiology of epileptic syndromes and their distribution across ages, gender, geographic distribution, costs and handicaps. Therefore, accurate epilepsy studies with well-defined epidemiological methods are necessary to verify the existing data and to determine the dimensions of the impact and burden of epilepsy in Libya.

However, one study reported higher prevalence in males but similar rate in middle-age and older individual. Idiopathic epilepsy represented 82.6% while symptomatic epilepsy was considered 18% (Sridharan et al. 1985).

2.7.3. Epilepsy and toxoplasmosis

Toxoplasmosis has recently been associated with some neurological disorders, particularly schizophrenia (Torrey & Yolken 2003) (Emelia et al. 2012) (Esshili et al. 2016), depression and bipolar disorder (Benazzi 2007) (Prandota 2009) (Duffy et al. 2015). However, evidence for causal relationships remains limited.

Over the past decade there has been an increasing interest in the possibility of an association between Toxoplasma gondii infection and epilepsy of unknown origin. Some studies focused on toxoplasmosis as a possible cause of cryptogenic epilepsy. Others looked into a relationship between toxoplasmosis and seizures of all types. In a search for comparative studies on the relationship between toxoplasmosis and cryptogenic epilepsy published until to date, we identified a total number of thirteen case-control studies that were carried out in 6 different countries or regions: USA,

Egypt, Iran, Turkey, Israel and some countries of Sub-Saharan Africa (Figure 16). Resumed information is available in tables 2 and 3.

2.7.3.1. Controlled studies

Two studies have been carried out in Turkey. Yazar et al. (2003) reported that the percentage of seropositivity to IgG against *T. gondii* was higher in 50 patients diagnosed of cryptogenic epilepsy (52%) than in 50 patients with symptomatic epilepsy and 50 healthy controls (22% and 18% respectively) (Yazar et al. 2003). This result is contrary to that reported by Akiol et al. (2007), who didn't find differences in prevalence of *T. gondii* infection between 100 patients with cryptogenic epilepsy and 50 healthy controls (Akyol et al. 2007).

Stommel and his colleagues (New Hampshire, U.S.A., 2001) compared 22 adults with cryptogenic epilepsy and 23 healthy adults as controls. They used qualitative ELISA for detecting IgG anti *T. gondii* and assessed optic density readings as a surrogate marker of IgG titers. They found that 75% of subjects with cryptogenic epilepsy, had antibody titers higher than median for controls. Authors concluded that increased anti-Toxoplasma antibody titers indicate that chronic *T. gondii* infection may be a cause of cryptogenic epilepsy (Stommel et al., 2001).

igure 15: Countries with studies about *Toxoplasma gondii* and epilepsy.

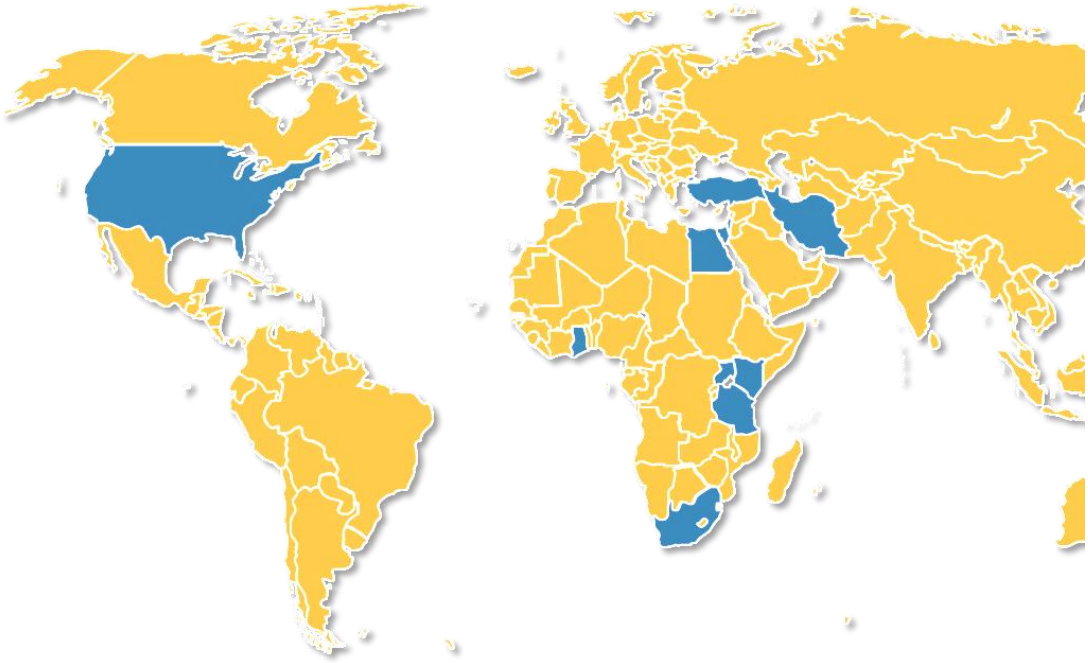


Table 2: Description of data extracted from the previous studies investigating an association between toxoplasmosis and epilepsy.

Authors	Region	Type of study	OR (95% CI)	P-value
Potasman et al. 1995	Israel	Hospital based	2.36 (0.91-6.08)	NS: 0.11
Stommel et al. 2001	USA	Hospital based	2.62 (0.72-9.54)	NS:0.075
Yazar et al. 2003	Turkey	Hospital based	5.35 (2.15-13.30)	<0.001
Akyol et al. 2007	Turkey	Hospital based	1.80 (0.80-4.05)	NS: 0.2
Zibaei et al. 2011	Iran	Hospital based	3.33 (1.03-10.78)	<0.05
Allahdin et al.2015	Iran	Hospital based	0.30 (0.2-0.6)	<0.001
El-Tantawy et al 2013	Egypt	Hospital based	2.01 (1.01-3.73)	<0.05
Eraky et al. 2016	Egypt	Hospital based	6 (1.19-30.10)	<0.05
Kamuyu et al 2014	sub-Saharan Africa	Community	1.28 (1.04-1.56)	<0.05
Ngugi et al 2013	sub-Saharan Africa	Community	1.39 (1.05-1.84)	<0.05

There are three studies in Iran about the rate of *Toxoplasma gondii* infection in epileptic patients. Zibaei et al. (2011) compared the rate of Toxoplasma infection in 85 epileptic patients with 85 healthy controls in west of Iran; they reported higher rate of the infection in epileptic patients (14.1 %) compared to the healthy controls (4.7 %) (Zibaei et al. 2011). In contrast, results of a study from south of Iran (Allahdin et al. 2015) indicated lower incidence of Toxoplasma infection in epileptic patients than in healthy controls (14.2 % versus 30.4 %). A more recent study by Babaie et al. (2017), estimated that the seroprevalence of Toxoplasma infection in Iranian epileptic patients (n=414), was not different from patients with non-epileptic neurologic disorders (n=150) and healthy controls (n=63); they didn't find significant differences in antibody titers (Babaie, Sayyah, Gharagozli, et al. 2017).

In Egypt, El-Tantawy et al. (2013) compared the frequency of *anti-Toxoplasma* seropositivity in 132 children diagnosed of cryptogenic epilepsy, aged 5 to 14 years, and 60 age-matched controls. They found that prevalence of *T. gondii* infection in children with cryptogenic epilepsy was 60.6% and 43.3% in controls ($p < 0.001$) (Labeeb El-

Tantawy et al. 2013). Similar findings were reported by Eraky et al. (2016), when they compared the rate of antibodies against *Toxocara* and *Toxoplasma gondii* among 40 children with cryptogenic epilepsy, 30 with non-cryptogenic epilepsy and 20 healthy control children. Their results showed that the rate of anti-*T. gondii* IgG seropositivity was higher among children with cryptogenic epilepsy (20%) than among children with non-cryptogenic epilepsy (0%) and healthy controls (10%) (Eraky et al. 2016).

Table 3: Summary of prevalence rates in previous studies investigating an association between toxoplasmosis and epilepsy.

Author		Epileptics		Controls		
	Age	Nº	Toxo %	Nº	Toxo %	p value
Potasman (1987-91, Israel)	1–15	52	19.2%	109	9%	NS
Stommel (1997-99, USA)	Adults	22	75%	23	56.5%	NS
Yazar (1999-2002, Turkey) - Cryptogenic - No cryptogenic	Adults	50 50	54% 22%	50	18%	<0.001
Akyol (2003, Turkey)	11-64	100	31%	50	20%	NS
Zibaei (2010, Iran)	7-62	85	14.1%	85	4.7%	<0.05
Alladin (2015, Iran)	2-39 yr	141	14.2%	144	30.4%	NS
Babaie (2016, Iran) - Epileptics - Neurological deficit no epil.	18-64 yr	414	35.3%	63 150	38.1% 34.7%	NS
El-Tantawy (2013, Egypt)	1-14 yr	132	60.6%	60	43.3%	<0.05
Eraky (2016, Egypt) - Cryptogenic epilepsy - Secundary epilepsy	9m-18 yr	40 30	20% 0%	20	10%	<0.05
Naglaa Fathy (2016, Egypt) - Depression - Cryptogenic epilepsy - No cryptogenic epilepsy	2-46 yr	 72 40	 34.7% 2.5%	60 118	11.7% 20.3%	<0.05
Ngugi (2007-11, SSA)	≥18 yr	555	47.7%	692	44.4%	<0.05
	<18 yr	416	27.6%	599	20.5%	NS
Kamuyu (2014, SSA)	0- ≥50	986	39.1%	1313	35.4%	<0.05

Toxo %: Percentage of IgG anti-Toxoplasma gondii positive. SSA: sub-Saharan Africa

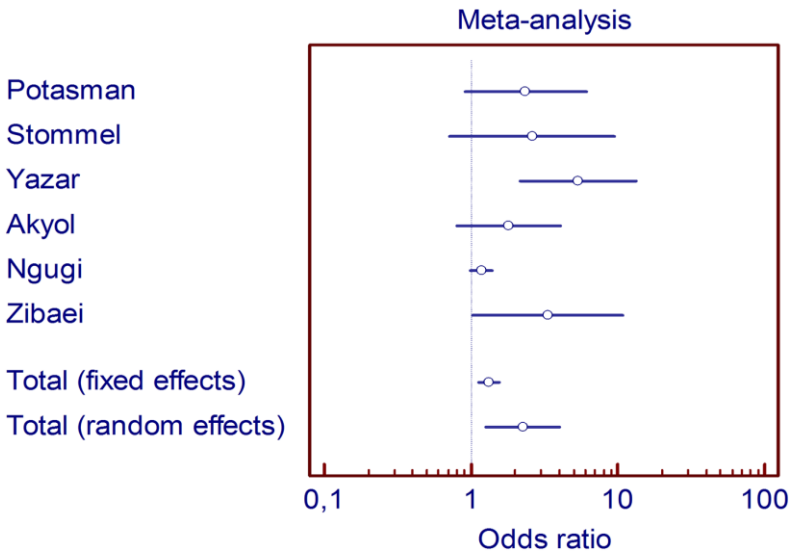
Ngugi et al. (2007), conducted large population-based cross-sectional and case-control studies to assess the prevalence and risk factors of active convulsive epilepsy in African patients. Study was performed across five centres in five sub-Saharan African countries (Kenya, South Africa, Ghana, Uganda and Tanzania). Anti-*T. gondii* seroprevalence in children (age <18 years) was not different between controls (25% of 599 tested) and epileptics (27.6% of 419 tested). But exposure to *T. gondii* in control adults (44.4% of 692 tested) was slightly lower than in epileptic adults (47.7% of 555 tested; $p<0.05$) (Ngugi et al. 2013). Kamuyu et al. (2014) studied exposure to multiple parasites and its relation to epilepsy in a case-control study. Study subjects were recruited in the same centres previously used by Ngugi, at five sub-Saharan countries. Seroprevalence of toxoplasma gondii infection was slightly higher in epileptics than in controls (39,1 % of 971 vs. 35.4 % of 1291) (Kamuyu et al. 2014).

2.7.3.2. Systematic reviews and meta-analysis

There are two meta-analysis in medical literature about the relationship between Toxoplasmosis and human epilepsy, made by Palmer (2007) and Ngoungou (2015). They found consistent higher rates of toxoplasmosis among epileptic patients than in control groups. The first meta-analysis was based on only three studies and found an Odds Ratio (OR) of 4.80 (95% CI 2.60-7.80) (Palmer 2007). Second meta-analysis based on six controlled studies found a lower OR of 2.25 (95% CI 1.15–3.93) (Ngoungou et al. 2015). Both authors concluded that there is a possible link between cryptogenic epilepsy and chronic toxoplasma infection.

However, the relationship between epilepsy and Toxoplasmosis has not been fully demonstrated. Quet (2007) argued that evidence of association between epilepsy and toxoplasmosis has not been sufficiently investigated, and, to date, there is doubt about whether this implicates causality and draw definitive conclusions (Mac et al. 2007) (Quet et al. 2008).

Figure 16: Scatter plot of odds ratios for epidemiological correlation between toxoplasmosis and epilepsy.



Ngoungou EB, Bhalla D, Nzoghe A, Dardé ML, Preux PM (2015) Toxoplasmosis and Epilepsy-Systematic Review and Meta-Analysis. PLOS Neglected Tropical Diseases 9(2): e0003525. https://doi.org/10.1371/journal.pntd.0003525

2.7.3.3. Pathogenesis of neurological symptoms

From the digestive tract, *tachyzoites* are distributed via the blood stream, establishing the acute infection parasitemia (acute stage). Finally, *tachyzoites* localize to muscle and CNS, where they differentiate in *bradyzoites* and multiply inside cells forming *tissue cysts* (chronic stage).

Latent infection (*bradyzoites*) is thought to be asymptomatic, because it is controlled by the intact immune system (Frenkel 1988). When the immune system becomes suppressed, i.e. AIDS, *bradyzoites* are reactivating, converting into rapidly proliferating *tachyzoites* that may cause lethal encephalitis if untreated. Alternatively, cyst rupture and formation of new cysts may be a constant process, even in immunocompetent individual (Feustel et al. 2012; Montoya & Liesenfeld 2004).

2.7.3.4. Possible mechanisms for epileptogenesis in *T. gondii* CNS infection

One can postulate how a dormant CNS infection such as *T. gondii* could cause epilepsy. Epileptic discharge may occur with a membrane defect leading to instability or

abnormalities of potassium, calcium channels or GABA inhibitory system (Palmer 2007).

In vitro infection of cultured CNS cells show the primary infection of T. gondii in astrocytes, microglia and neurons (Mammari et al. 2014). But there is clear tropism of *T. gondii* for neurons in which encysted parasites were exclusively found (Cabral et al. 2016). This is not due to selective invasion; there may be unknown properties of neurons that promote cyst development.

In the mouse model, there is evidence of focal inflammation with disrupted *tissue cysts* (Sims & Hay 1995). *Toxoplasma tissue cysts* mature slowly and have been shown to eventually lyse in the immunocompetent host, producing microscopic scars (glial nodules) (Bertoli et al. 1995).

Intermittent increase of anti-Toxoplasma IgG levels in some patients suggests that the *bradyzoites* may reactivate and subsequently produce an antibody response (Frenkel 1988). Reactivation of the cysts could depolarize cells being exited or entered. When *T. gondii* has entered the cell, it is then able to hijack the host cell Ca^{2+}-pump. Before *T. gondii* leaves the host cell, there is an increase in the intracellular Ca^{2+} concentration, which allows *T. gondii* to escape and is followed with lysis of the host cell. Cell lysis will also cause microglial formation (Frenkel & Escajadillo 1987).

Toxoplasma encephalitis is characterized by many foci of enlarging necrosis and microglia nodules that calcify and lead to radiological changes and can rise to epileptic discharges (Cook et al. 2000) (Dubey et al. 1998). The formation of scar tissue has been suggested to be one of the main theories for the cause of epilepsy (Prayson & Frater 2002).

Neurons infection by *Toxoplasma gondii* promote changes in regulation of neurotransmitters. Stibbs described changes in neurotransmitter concentration in the brains of chronically infected mice, with reduction in serotonin and norepinephrine, and an increase in total dopamine (Stibbs 1985). Other authors also found increased dopamine turnover in in chronically infected mice (Prandovszky et al. 2011) (Ihara et al. 2016). It has been observed in vitro that dopamine is able to increase parasite proliferation (Strobl et al. 2012). Treatment of rats and mice with dopamine D1 an D2

receptor antagonists inhibit the establishment of behavioral changes after experimental T. gondii infection (Webster et al. 2006).

T. gondii infection decreases the threshold of clonic seizures produced by infusion of pentylenetetrazole in mice. This increased susceptibility to seizures was confirmed in acute and chronic phases of *T. gondii* infection (Babaie et al. 2017). These authors demonstrated that increased seizure susceptibility was reversed with pre-treatment with dopamine inhibitors. Treatment of toxoplasmosis with trimethoprim/sulfamethoxazole definitively restored the susceptibility for seizures to the level of uninfected mice (Babaie et al. 2017).

HIPOTHESIS AND OBJECTIVES

3. HYPOTHESIS AND OBJECTIVES

A link between acquired epilepsy and toxoplasmosis was suggested by some authors in recent years. But most studies checking this possible relation are small and some shows contradictory results.

Cryptogenic epilepsy lacks of known aetiology, but it is suspected to be secondary. Patients with cryptogenic epilepsy have not known cause of epilepsy after considering his personal and familial antecedents, habitual neurological studies and clinical data. If *Toxoplasma gondii* infection can cause epilepsy, probably patients with epilepsy secondary to toxoplasmosis will be catalogued as cryptogenic epilepsy. It is less probable that patients with epilepsy secondary to toxoplasmosis will have other known cause of epilepsy or genetic epilepsies.

3.1. Hypothesis

We hypothesized that *Toxoplasma gondii* infection can cause epilepsy in children. If toxoplasmosis is related to development of epilepsy, children with suspected cryptogenic epilepsy in Libya will have a higher frequency of *Toxoplasma gondii* infection than non-epileptic children. Children with suspected cryptogenic epilepsy will also have a higher frequency of *Toxoplasma gondii* infection than epileptic children with known causes of epilepsy.

3.2. Objectives

3.2.1. Main objective

The main objective of the present work is to determine if acquired *Toxoplasma gondii* infection is related to the development of cryptogenic epilepsy in Libyan children.

To assess this question, we will determine:

- If children with suspected cryptogenic epilepsy have higher frequency of *Toxoplasma gondii* infection than non-epileptic children.

- If children with suspected cryptogenic epilepsy have higher frequency of *Toxoplasma gondii* infection than children with symptomatic epilepsy.
- If children with suspected cryptogenic epilepsy have higher anti-*Toxoplasma gondii* IgG titers than controls.

3.2.2. Secondary objectives

- To assess possible relations of *toxoplasma gondii* infection with seizure types and degree of control of epilepsy.
- To estimate the prevelance of *Toxoplasma gondii* infection in children with epilepsy.
- To estimate the prevelance of *Toxoplasma gondii* infection in non-epileptic children selected as controls.
- To identify risk factors and possible routes of infection associated with *Toxoplasma gondii* infection and quantify their influence.

SIGNIFICANCE OF THE STUDY

4. SIGNIFICANCE OF THE STUDY

In Libya, there is not much evidence-based information on *T. gondii* infections among high-risk groups. Our data will not provide a complete picture of prevalence of *T. gondii* infections in the entire population of Libya; but this is a new area of research and may provide the basis for extended future studies.

Toxoplasmosis is a preventable disease. Understanding the possible association between toxoplasmosis and epilepsy could be an important step in future effort to prevent epilepsy in low resource settings countries and subsequently reduce of the morbidity and financial expenditure for the treatment of patients.

Identification of main transmission routes of the infection in Libyan children will be useful for planning optimal measures against infection. In the future, it may allow for the implementation of appropriate public health policies, through increased awareness and better control of toxoplasmosis.

If possible links between *Toxoplasma gondii* infection and epilepsy are confirmed, appropriate strategies can be designed to include this knowledge in the treatment of patients. Diagnosis of toxoplasma infection can be included in the routine study of epileptic patients. The information available in the experimental models on the most effective drugs to deal with toxoplasmosis-related seizures, could be transferred to the clinical care of the patients. Finally the treatment of toxoplasmosis could be considered in those patients in whom this infection may play an etiological or aggravating role of their epilepsy.

METHODS

5. METHODS

5.1. Introduction to the study

We have designed a controlled study for epileptic patients attending the National Center for the Treatment of Epilepsy in Tripoli, Libya. Controls were selected from a group of non-epileptic patients who attended the general paediatrics outpatient clinic at the Tripoli Medical Centre hospital during the same period. The total number of participants was 298. Blood samples were collected and tested for the presence of antibodies against Toxoplasma using an immunoenzymatic assay. Data on risk factors for T. gondii infection were obtained through personal interview and review of medical records.

5.2. Study design

A comparative hospital-based study was undertaking. The prevalence of anti-*toxoplasma gondii* antibodies has been estimated in children with cryptogenic epilepsy and in two control groups, patients with secondary epilepsy and non-epileptic children.

5.2.1. Study location, selection of subjects

The Libyan health care system consists of public (funded by the government) and private health services. Public hospitals provide out-patient and in-patient services for the majority of children in our community. The setting of the study took place at the capital of Libya, Tripoli, located at west region of Libya, on the Mediterranean coast.

Epileptic patients were selected from the National Center for the Treatment of Epilepsy in Tripoli city, which has an outpatient clinic for admission and follow up of patients with epilepsy. Children with a wide age range attend these clinics with their parents. Patients regularly receive their medication from the hospital pharmacy and a routine assessment of their condition is carried out periodically by specialists.

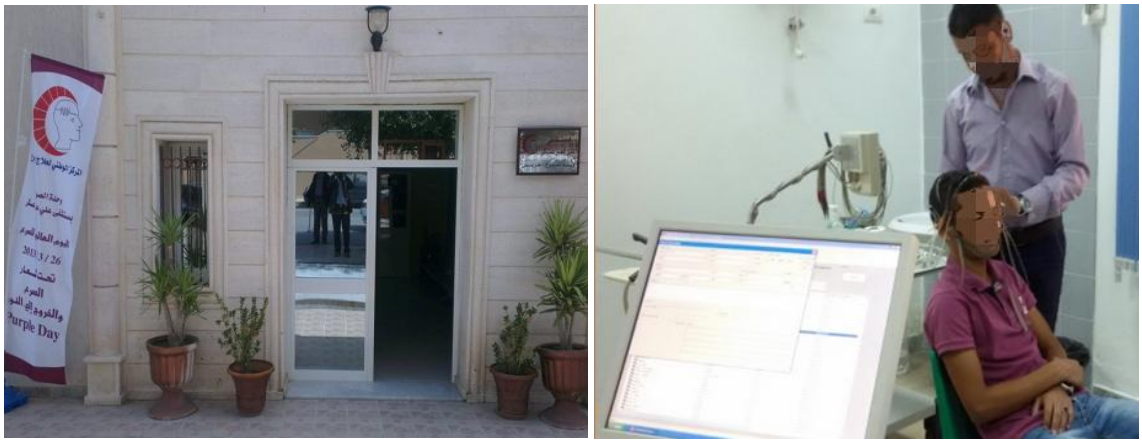
The controls were selected from the Tripoli Medical Center which is the largest hospital in the west region of Libya with 1200 beds.

5.2.1.1. The National Center for the Treatment of Epilepsy

The National Center for the Treatment of Epilepsy was built in 1997. It is one of the largest referral centres for patients with epilepsy in Libya. About 17000 patients including adults and children are registered with the center. About 9000 patients (52%) are from the Tripoli area and the rest are from urban and rural areas of western Libya. The records indicate that about 5000 of these patients probably have not had recurrences of epilepsy and could be considered cured.

It has six outpatient clinics and eight specialist doctors in addition to twelve workers and nurses. There is a clinical laboratory to perform basic analytical determinations, including the determination of serum drug levels and also a separate EEG laboratory. The pharmacy distributes free anti-epileptic drugs to patients. It has an emergency room and IQ room to detect and follow the intellectual disability of patients. The center does not have radiology services to perform CT or MRI, therefore, the patient has to run these tests in public hospitals or private clinics.

Figure 17: The National Center for the Treatment of Epilepsy



Main gate *EEG lab*

Figure 18: The National Center for the Treatment of Epilepsy

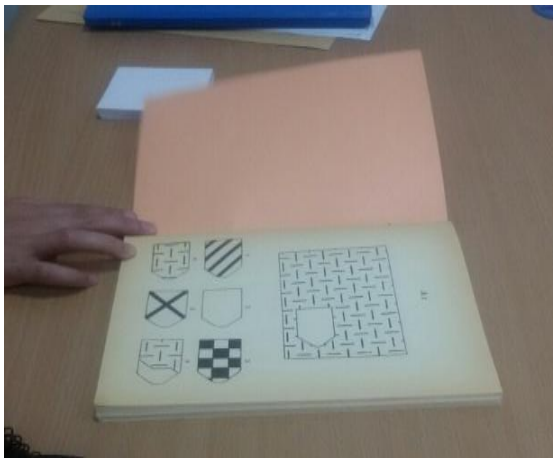

Patients Waiting Sala


Clinical laboratory *Administrative archive*

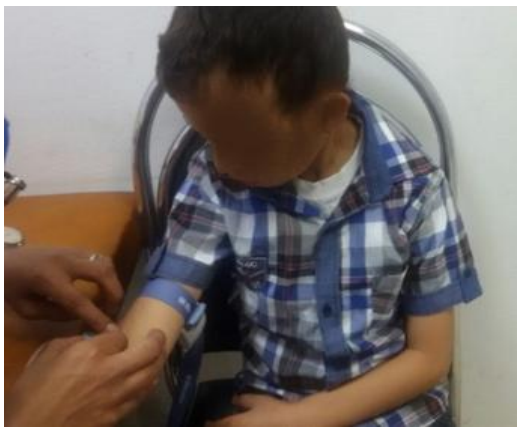

Centre pharmacy

Figure 19: The National Center for the Treatment of Epilepsy

Observation room



IQ room



Epileptic Patient follows up

5.2.1.2. Tripoli Medical Center

The controls were selected from Tripoli Medical Center which is the largest teaching hospital in the west region of Libya with 1200 beds. It serves as a major reference centre for patients from the western division with a population of nearly two million. This centre offers all specialty services with advanced laboratory facilities. The control group of children were selected either consecutively from paediatric out-patient Clinics at Tripoli Medical Centre or opportunistically from children who had been admitted to the hospital in the Paediatric Department at the same hospital.

Figure 20: The Tripoli Medical Centre

5.2.2. The study participants and inclusion criteria

Study design considered three groups of children:

- Group A: children with epilepsy (150 cases) divided in two subgroups.
    - A1: Suspected cryptogenic epilepsy (group name: Cryptogenic).
    - A2: Secondary epilepsy (group name: Symptomatic).
- Group B: children without epilepsy (150 cases).

Common inclusion criteria for all groups were:

- Aged from 6 to 18 years old.
- Absence of congenital syndromes/malformations.
- Parents and children acceptance to participate in our project.

5.2.2.1. Group A: children with epilepsy

Children with epilepsy were sequentially recruited at The National Center for the Treatment of Epilepsy, Tripoli, Libya. Recruitment of these patients was made from March to July of 2014, when the patients arrived to follow up.

Patients with epilepsy who were candidates to be recruited for the study had a preliminary assessment of their suitability, excluding those with more probable genetic causes of epilepsy. If they were to participate in the study, they were assigned to the group of patients with cryptogenic or symptomatic epilepsy according to data collected from their clinical records and interview. The inclusion and exclusion criteria for both groups are described below.

Finally, two of the 150 epileptic patients initially recruited were excluded because missed serum samples.

5.2.2.1.1. Group A1: children with suspected cryptogenic epilepsy (Cryptogenic)

Group-A1 finally includes 92 patients

Specific inclusion criteria of this group were:

- Epileptic children presented with recurrent epileptic fits with unknown aetiology.
- All types of partial and/or generalized seizures
- Patients had no past history of head trauma, brain surgery, meningitis or encephalitis.
- Normal brain Magnetic Resonance Imaging Scan (MRI) and/or normal–Computed Tomography Scan (CT).

Exclusion criteria were:

- Patients with seizure onsets under 6 years of age were excluded from this group.
- Any disease with known relation with epilepsy.
- Past history of perinatal hypoxia, difficult birth, head trauma, brain surgery, previous meningitis, encephalitis.
- Abnormal brain Magnetic Resonance Imaging Scan (MRI) or abnormal–Computed Tomography Scan (CT).
- Family history of seizures.

5.2.2.1.2. Group A2: children with secondary epilepsy (Symptomatic)

Total of 56 patients suffering from symptomatic epilepsy were included in Group A2.

Specific inclusion criteria of this group were:

- Presented with recurrent epileptic fits with known aetiology.
- All types of partial and/or generalized seizures.
- CT or MRI severe abnormalities that justify patient's epilepsy.

Exclusion criteria:

- Congenital malformations o congenital syndromes.
- Family history of seizures.

5.2.2.2. Group B: non-epileptic children

The 150 controls were enrolled from children attending the Tripoli Medical Centre for causes other than epilepsy. Most of these would include patients receiving a venepuncture for medical reasons and an aliquot of this sample would be requested for use as the control serum. Control patients selection was stratified by age groups (6-9 yr, 10-13 yr, 14-17 years) for matching age distribution in Group A.

Inclusion criteria of this group were:

- Aged from 6 to 18 years old.
- Neurologically normal
- No history of seizures.

Exclusion criteria

- History of seizures in their first degree relatives.
- Congenital malformations o congenital syndromes.

5.2.3. Ethics approvals and consent

The researcher obtained permission from the head department of National Epileptic and Centre Tripoli Medical Centre before making the field trip.

A consent form added, that assuring participants that their confidentiality were respected and their anonymity guaranteed. In the current study, the participants were asked to indicate their name as an essential step which should be taken into serious consideration which was limiting the possibility of having their blood test results mixed

up with other patients. Participants were assured that either their acceptance or declining to participate in this study, would not affect their relationship with health center staff, their physicians or the care the children would receive. In addition, it was reported that if the child had *Toxoplasma gondii* infection, it will not be treated.

Epileptic children have many barriers related to the socioeconomic circumstances of their families and social stigma. Particularly women are very conservative and reserved, being difficult to conduct interviews. During the interviews, I had to assure the patient and her parents that their answers were completely anonymous. This allowed them to feel comfortable and relaxed enough to speak freely.

An information sheet was given in Arabic describing how this infection is acquired and the measures that can be taken to prevent it.

5.2.4. Reporting of study result

Usually parents of children wished to know the test results of their children. The results were copied and sent to clinical consultants in both the National Center for the Treatment of Epilepsy and the Infectious Disease Department in Tripoli Medical Center to allow them to inform parents who had requested the test result.

5.2.5. Study procedure

5.2.5.1. Variables and questionnaire survey

The questioner was available in Arabic and English form. To ensure the cultural appropriateness of the questions and to guarantee that each question was fully understood, the questionnaire was designed and tested for its Arabic and English. All questionnaires had unique numerical identifiers. English questionnaires are shown in Appendix (Section 11.2).

Patients questionnaires were filled out on the day blood samples were obtained. For the parents who didn't know how to read or understand, the researcher read the questions and explains to them in simple way.

The Questionnaire consists of three parts. In the first part questions are about the general socio demographic characteristics of the child and risk factors of toxoplasmosis,

the second part had questions directed at determining if they experienced perinatal complications. The third, part was about child development.

Data recorded for epileptic children and controls included:

- The Socio-demographic data including age, gender and residence.
- History of blood transfusions.
- Children habits and behaviours, including the kind of meat consumption (raw or undercooked), source of drinking water and milk, raw vegetables, personal hygiene and contact with soil, cats and dogs.
- Perinatal complications.
- Neurological development, such as age of walking independently, age of speaking and speak disorders, intellectual disabilities and education and academic success.

For epileptic patients:

- Medical antecedents, such as birth characteristics and complications, diseases, accidents and hospitalizations.
- Neurological development, neurological deficits, intellectual capacity (IQ), visual and hearing impairments.
- History and type of epilepsy including; age of onset, frequency and distinguishing features.
- Cognitive development was assessed by psychologist using cognitive tests and recorded from medical file.
- Results of brain MRI, CT and EEG (electroencephalogram).
- Antiepileptic drugs usage and control of seizures.

These data were obtained from interview and reviewing medical files for each patient.

5.2.5.2. Interviews

Interviews were completed by personal interview by the investigator. The procedure took about 10-15 minutes. For epileptic and control patient, this was done after clinic attendance.

The medical records were then reviewed for completing medical information as EEG reports, brain imaging study reports and treatment.

Figure 21: Completing patient records

5.3. Toxoplasma gondii serology

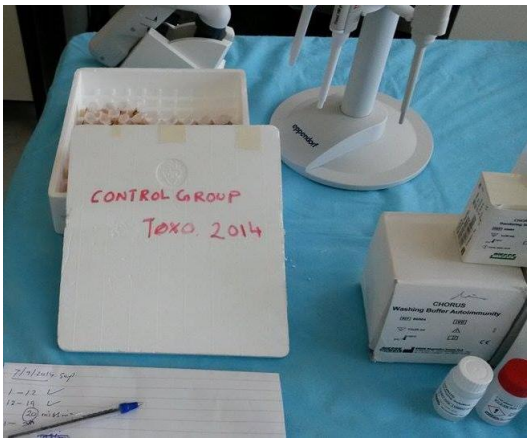
5.3.1. Serum collection an preservation

Supplies for blood samples were available in both centres. A 5 ml sample of blood was collected from each child by a qualified medical technician under sterile conditions with minimum discomfort to participants. The samples were collected in a sterile Vacutainer with no additives and centrifuged.

Serum was frozen and stored at -20ºC at the Tripoli Medical Center's Clinical Immunology Lab until analysed for detection of Toxo-IgG antibodies. Maximum time elapsed to analysis was 4 months.

Figure 22: Serum collection and preservation

Cases study samples

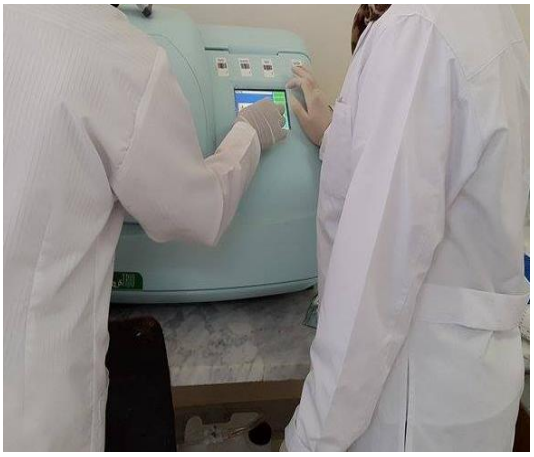


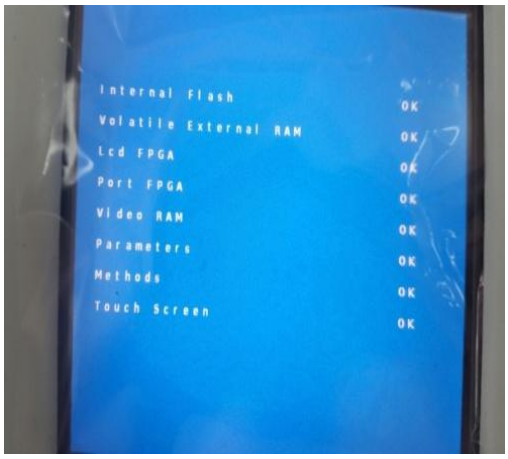
Control study samples

5.3.2. ELISA Assay Procedures

Serum samples were screened for anti-T. gondii IgG by ELISA using "Toxoplasma IgG kit" *(DIESSE Diadnostica Senese. SPA 53035 Monteriggioni (SI) Italy).* Procedure was conducted according to the manufacturer's instructions. Positive and negative controls were used with each series of anti *T. gondii* IgG. Results were obtained at 450 nm absorbance. All these procedures were performed by the investigator with assistance of a laboratory technician at the Immunology Laboratory in Tripoli Medical Center.

Figure 23: Lab training





Switch on instrument and Start-up program

5.3.2.1. Material Required

- Chorus Trio-81200 instrument.
- Toxo-IgG kits with control and calibrator.
- Sample rack.
- Freezer and refrigerator
- Micropipettes (50ul, 100ul & 1ml).
- Laboratory glassware: cylinders and test tubes.
- Disposable gloves.

5.3.2.2. Quantification of anti-Toxoplasma IgG Antibodies

Before starting the analysis, the Chorus system needs to setup the cleaning and washing buffer, then calibration procedure. Test is developed in individual disposable devices that include all necessary reagents (Figure 24).

After fixing all devices in the Chorus instrument, 50µl of collected serum is added in the device.

There are three steps involved to complete this enzymes immune assay:

1. Conjugates with incubation (30 minutes).
2. Wash three times.
3. Stop reaction and quantification.

System spent approximately 140 minutes to finish results for 29 samples. The results were released automatically through the thermal printer in international units (IU/ml).

Figure 24: Test device.

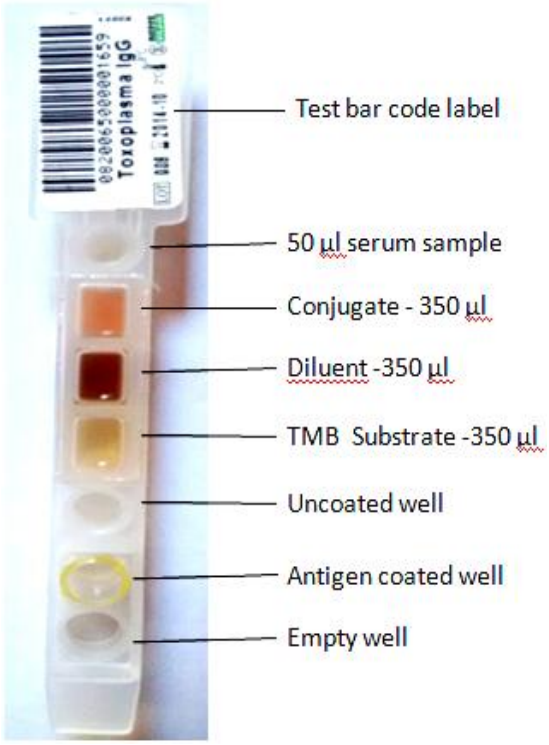
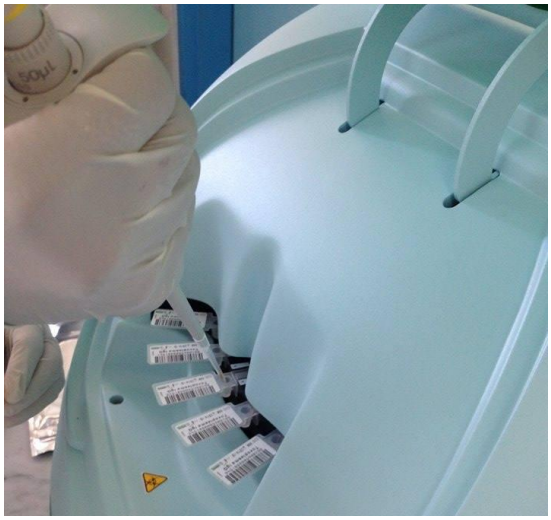


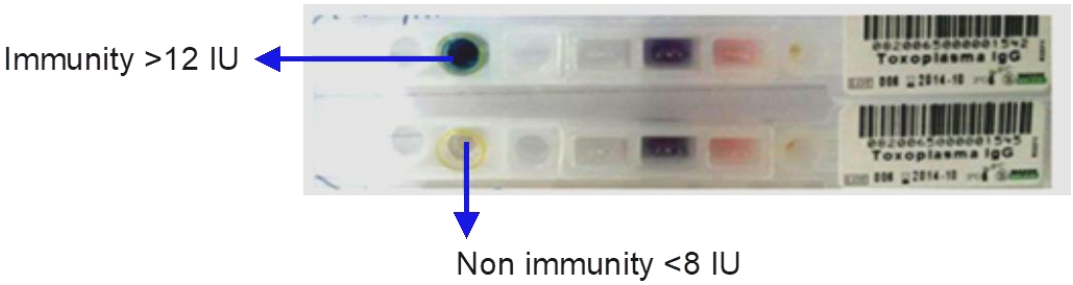
Figure 25: Device loading in tray



The degree of immunity of the test serum can be interpreted as follows:

- Values higher than 12 IU/ml were considered positive.
- Values lower than 8 IU/ml were considered negative.
- Values from 8 to 12 IU/ml are considered indeterminate or doubtful

Figure 26: Results interpretation



5.4. Data storage and handling

Information collected was entered into a database using a coding system to anonymize the data collected. Access to the data was restricted to the project staff. Questionnaires and laboratory results were kept secure by the principle investigator. Firstly, data were entered into MS Excel. Data were checked manually to look for inconsistencies and outliers. Exploratory analysis was made using statistical software for final detection of mistakes..

5.5. Statistical analysis

5.5.1. Hypothesis testing

5.5.1.1. Bivariate data analysis

Percentages of qualitative variables were compared by Chi-squared or Fisher's exact test. Ordinal variables were compared with Mann-Whitney test or Kruskal-Wallis ANOVA. When applicable, continuous variables were compared by t-test or ANOVA.

Welch's test was used for comparisons of continuous variables if assumption of homogeneity of variances was rejected.

5.5.1.2. Multivariate data analysis

Multivariate data analysis of risk factors was made with binomial logistic regression, with backward stepwise variable selection. Model fit was confirmed by ROC curve analysis of predicted values.

5.5.1.3. Statistical significance level

For null hypothesis significance testing, a threshold value for $\alpha = 0.05$ cut-off was chosen. The null hypothesis was rejected when $p < 0.05$. Two-tailed contrasts were used for all comparisons.

5.5.2. Data presentation

Categorical data are shown as percentages. Continuous variables are resumed as mean ±95% confidence interval (C.I.). Odds ratios (ORs) were shown with 95% confidence interval.

5.5.3. Statistical software

Statistical software programs used in this study were:

- IBM SPSS Statistics: IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.
- STATISTICA: StatSoft, Inc. (2014). STATISTICA (data analysis software system), Version 12.

RESULTS

6. RESULTS

6.1. General introduction

This chapter presents the results of the study. It begins describing the demographic characteristics and descriptive study of epileptic patients and control group. Third section shows results of toxoplasmosis serology, the analysis of socio-demographic and behavioural factors related with toxoplasma infection and relations of IgG anti-*T. gondii* results with study groups. The final section interprets the characteristics of epilepsy in studied patients and anti-*Toxoplasma* antibodies prevalence amongst patients and with different types of seizures.

6.2. Subjects in study

Were selected 300 participants (150 epileptic patients and 150 non-epileptic controls). Serum samples were missed in 2 epileptic patients. Finally, 298 cases were included in study.

6.2.1. Whole group characteristics

Their mean age was 11 ±3 years (range 6–17.5 years). Figure 27 shows age range and Table 4 shows distribution by age and sex groups.

The majority of participants live in rural and sub urban areas 117 (39%), and 96 (32%) respectively. The lowest proportion was urban area 85 (28%), see Figure 29.

Figure 27: Age distribution in study subjects

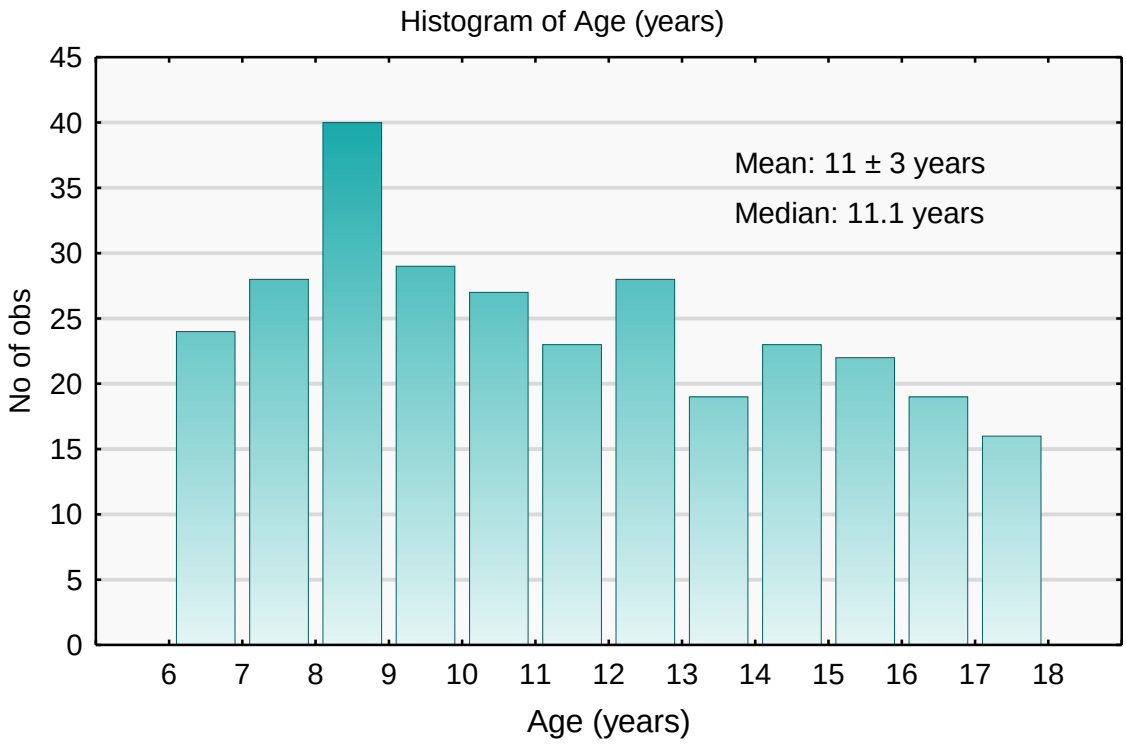


Figure 28: Gender distribution in study subjects

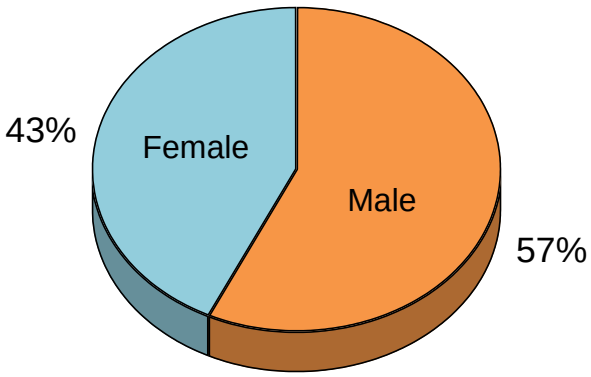
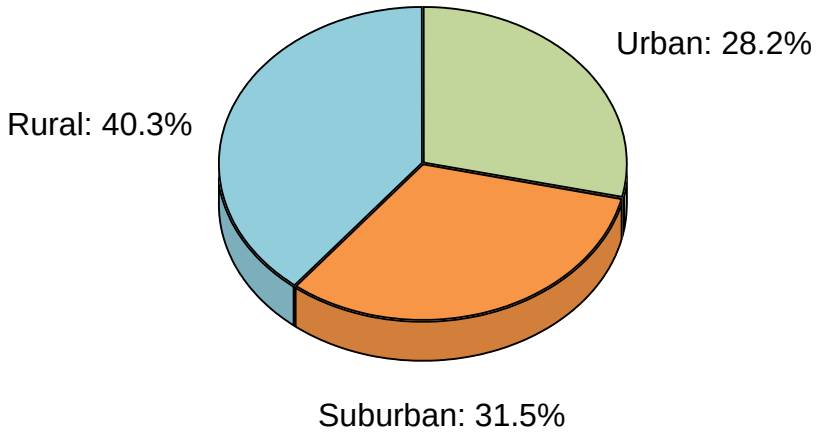


Table 4: Cross-tabulation of gender and age

	Age (years)			Total
	6-<10	≥10-<14	≥14	
Male (column %)	68 (56.2%)	57 (58.8%)	45 (56.3%)	170 (57%)
Female (column %)	53 (43.8%)	40 (41.2%)	35 (43.7%)	128 (43%)
Total (row %)	121 (40.6%)	97 (32.6%)	80 (26.8%)	298 (100%)

Percentage by columns

Figure 29: Residence



6.2.2. Epileptic patients: 148 cases

Epileptic patients with congenital syndromes, known genetics base epilepsy, chromosomal abnormalities and first or second degree relatives affected of epilepsy were excluded at baseline.

Included patients were initially classified into two groups, cryptogenic epilepsy and symptomatic epilepsy.

6.2.2.1. Cryptogenic epilepsy: 92 cases

These patients had normal brain MRI (91 cases) or CT (1 case). Cognitive assessment was made by psychologist technician and results are shown in Table 5. We lack of confirmation about what cognitive tests was performed in most of patients. Table 6 shows walking and speaking age in this group.

Table 5: Cognitive disability in patients with cryptogenic epilepsy

	Frequency	Percentage
Normal	31	33,7%
Mild intellectual disability	20	21,7%
Moderate intellectual disability	20	21,7%
Not tested	21	22,8%
Total	92	100%

Unknown test applied in most patients.

Table 6: Speaking and walking age in patients with cryptogenic epilepsy

	Minimum	Maximum	Mean	S.D.
Speaking age (months)	13	36	16,6	2,8
Walking age (months)	9	15	11,8	1,7

6.2.2.2. Symptomatic epilepsy: 56 cases

Most of these patients had severe neurological disabilities. 84% had abnormal brain MRI or CT (Table 7). Table 8 shows aetiology of brain damage and epilepsy in this group. Only 20% can walk. Some kind of speaking disability was present in 47 (84%). Table 9 and Table 10 show intellectual and speaking disabilities in this group.

Table 7: Aetiology of brain damage in patients with Symptomatic epilepsy

	Frequency	Percentage
Birth related	20	35.7%
Prematurity	13	23.2%
Meningitis	10	17.9%
Jaundice encephalopathy	3	5.4%
Sepsis	2	3.6%
Apnoea	2	3.6%
Other	3	5.4%

Table 8: CNS imaging in patients with Symptomatic epilepsy

	Frequency	Percentage
PVL +/- hydrocephaly	15	26.8%
PVL+SCL	9	16%
IVHg IV+/- hydrocephaly	9	16%
Hydrocephaly	5	8.9%
Abnormal basal ganglia	3	5.4%
Infarction	2	3.6%
Multicystic encephalomalacia	2	3.6%
Miscellaneous	2	3.6%
Normal	9	16%

PLV: periventricular leucomalacia ; SCL: subcortical lesion ; IVHg: intraventricular haemorrhage

Table 9: Cognitive disability in patients with Symptomatic epilepsy

	Frequency	Percentage
Mild intellectual disability	13	23,2%
Moderate intellectual disability	14	25,0%
Severe/very severe intellectual disability	29	51,8%
Total	56	100%

Unknown test applied in most patients.

Table 10: Speaking disability in patients with Symptomatic epilepsy

	Frequency	Percentage
Normal speech	9	16,1%
Speechless	11	19,6%
Mild dysarthria	14	25,0%
Severe dysarthria	22	39,3%
Total	56	100%

6.2.2.3. Comparisons between epileptic patients

6.2.2.3.1. Age and sex.

Studied patients with symptomatic epilepsy had lower age at onset of seizures (Table 11). Actual age was slightly lower in symptomatic epilepsy group.

Table 11: Comparison between cryptogenic and symptomatic groups for age and onset of seizure

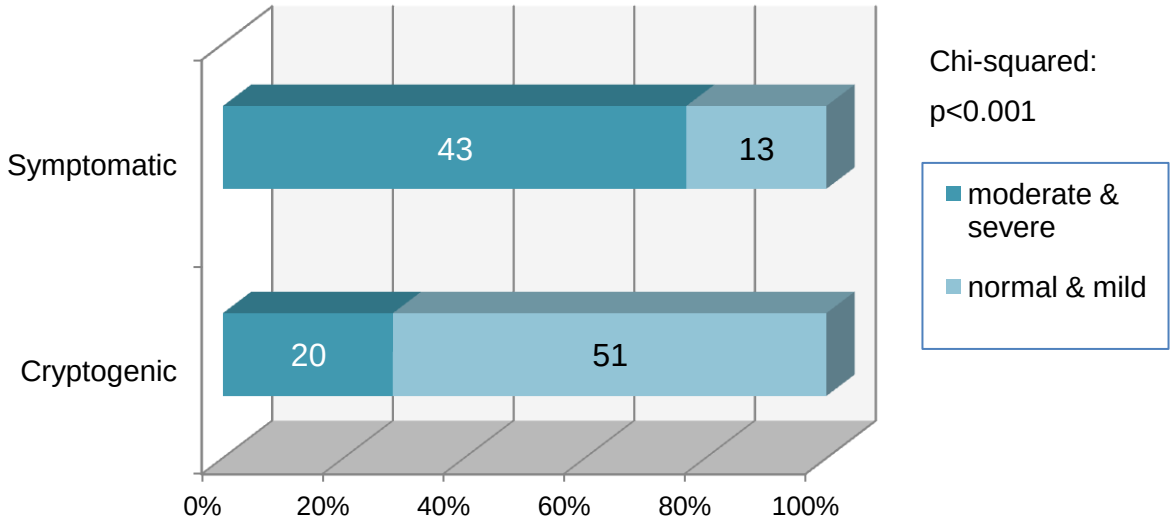
	Cryptogenic Mean ±SD	Symptomatic Mean ±SD	t-test p
Age	11.74 ±3.25	10.37 ±3.06	<0.05
Age at epilepsy onset (yr.)	8.16 ±2.63	3.86 ±2.41	<0.001

There were no differences in gender between epileptic subjects. Gender proportions were M: 58.7% / F: 41.3% in cryptogenic epilepsy patients, and M: 62.5% / F: 37.5% in symptomatic epilepsy.

6.2.2.3.2. Intellectual disability

Most of epileptic patients were evaluated for cognitive status (Table 5 and Table 9). Figure 30 resumes intellectual status of both groups of epileptic patients.

Figure 30: Intellectual disability in epileptic patients



6.2.3. Non-epileptic controls: 150 cases

Consecutive controls were selected at Tripoli Medical Centre General Paediatrics outpatient clinic and General Paediatrics hospitalization. These patients had no antecedents of seizures or any known neurological disease. Mean age was 11 years, SD 3.3 years. Were Males: 54% and Females: 46 %.

6.2.4. Demographic characteristics of study groups

Table 12 resumes demographic characteristics of study groups. There were no differences in age, sex and residence between study groups.

Table 12: Demographic characteristics of study groups

		Cryptogenic Epilepsy (N 92)	Symptomatic Epilepsy (N 56)	Non epileptic (N 150)	p-value
Age (years)	6-9	34 (37%)	28 (50%)	59 (39%)	NS
	10-13	30 (33%)	17 (30%)	50 (33%)	
	14-17	28 (30%)	11 (20%)	41 (27%)	
Gender	Male	54 (59%)	35 (63%)	81 (54%)	NS
	Female	38 (41%)	21(38%)	69 (46%)	
Residence	Urban	21 (23%)	18 (32%)	46 (31%)	NS
	Suburban	29 (32%)	15 (27%)	52 (35%)	
	Rural	42 (46%)	23 (41%)	52 (35%)	

Percentage by columns

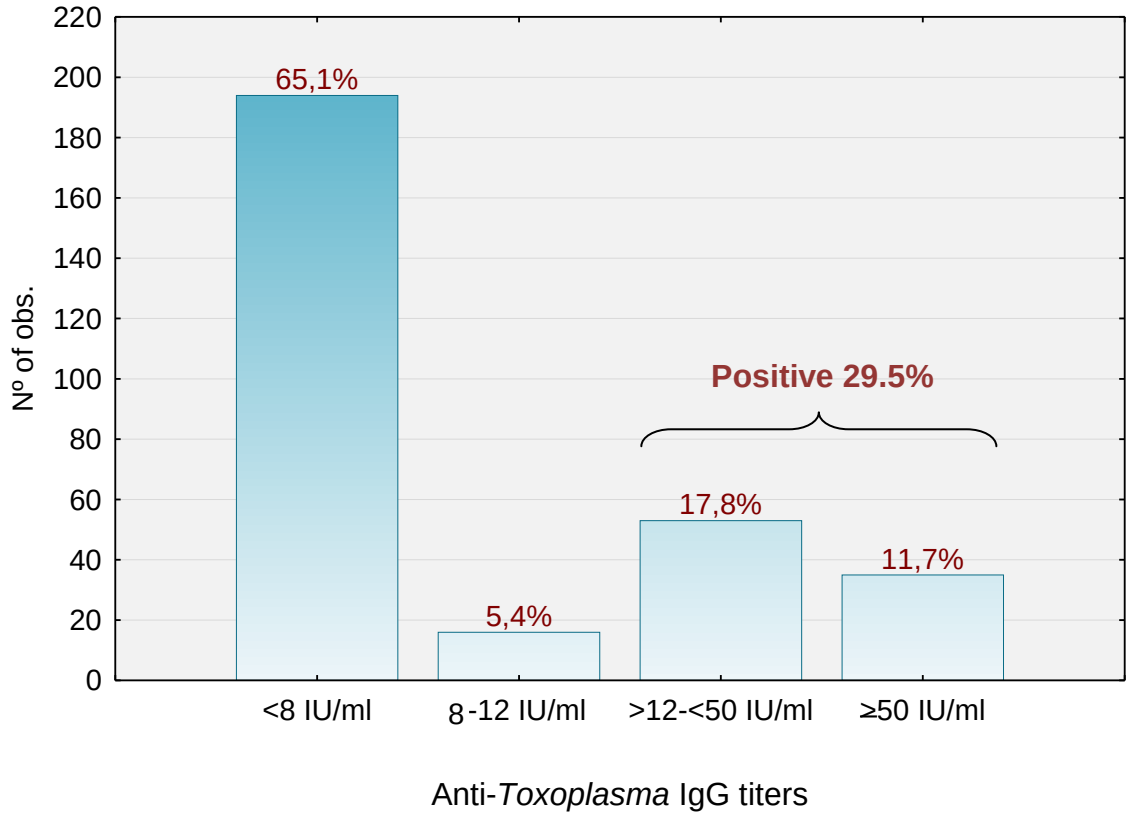
6.3. Toxoplasmosis serology

6.3.1. Prevalence of positive antibody titters

Serum samples were screened with enzyme linked immunoassay for the presence of anti-*Toxoplasma gondii* IgG.

Figure 31 shows anti-*Toxoplasma* IgG titers for whole group. Titers under 8 IU/ml are considered negative, between 8 and <12 IU/ml are doubtful and titters ≥12 IU/ml are positive. The prevalence of toxoplasma infection in these children was close to expected, with 29.5% of IgG positivity.

Figure 31: Anti-Toxoplasma IgG in epileptic children and controls



Titers <8 IU/ml are negative, 8-<12 IU/ml are doubtful and titters ≥12 IU/ml are positive.

6.3.2. Socio-demographic characteristics related to *Toxoplasma gondii* infection

6.3.2.1. Seroprevalence of toxoplasmosis according to age qroup

Epileptic children and controls were divided in two age groups (6-<12 and 12-<18 years). There were no differences in prevalence on infection or antibody titters between these groups (Figures 32 and 33). The prevalence of *Toxoplasma gondii* infection was 28% for children of 6- to <12 years old and 31% in the older group (p=NS).

There was no significant correlation between age and anti-*Toxoplasma* IgG titers (Spearman's rank order correlation coefficient= 0.07; p=NS).

Figure 32: Anti-Toxoplasma IgG antibodies by age groups

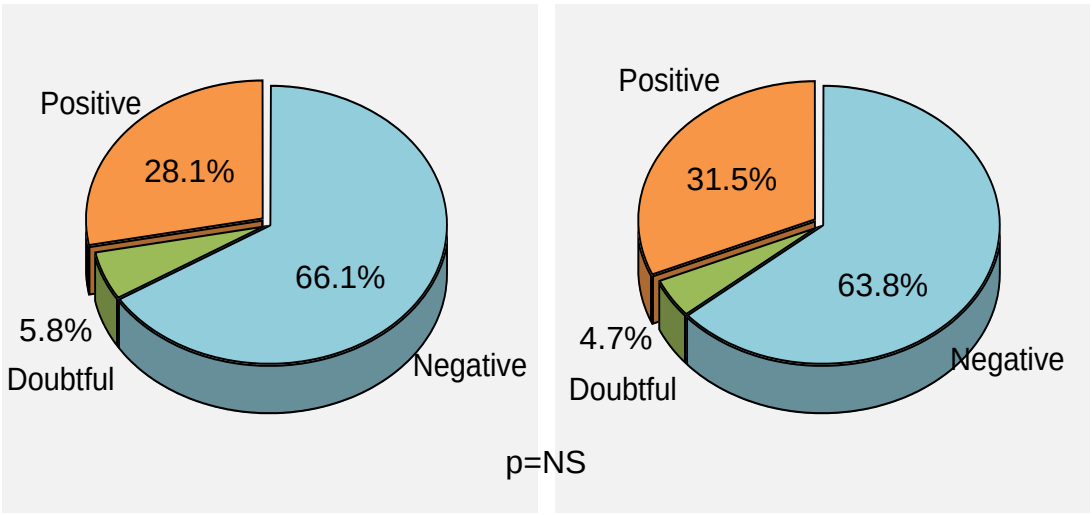
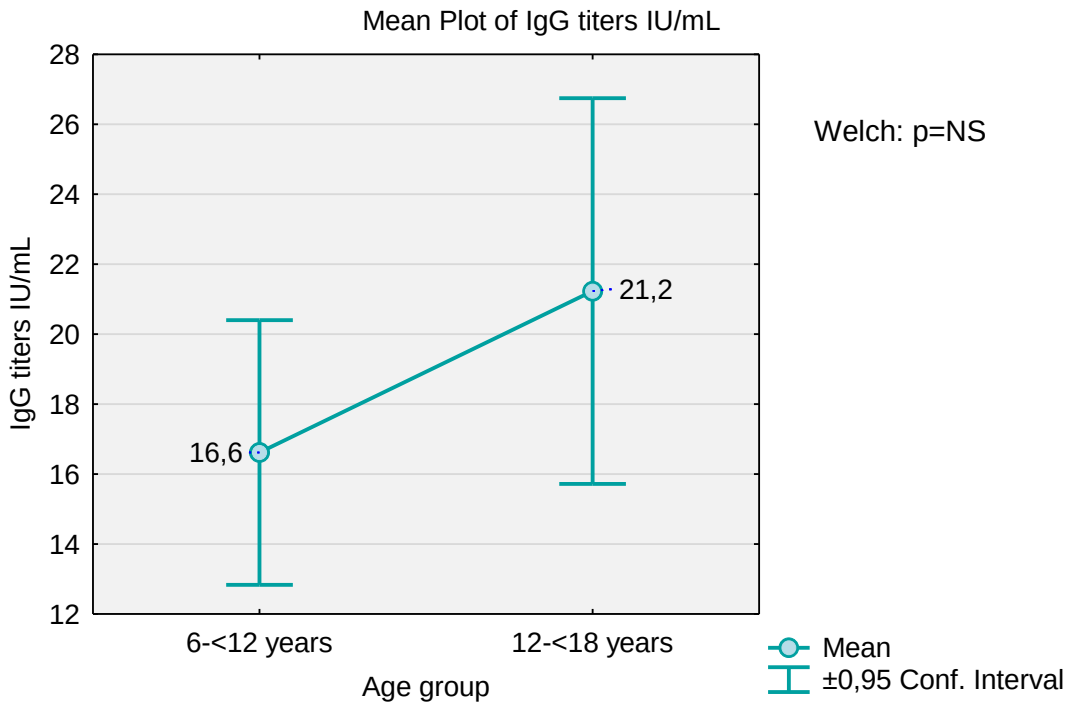


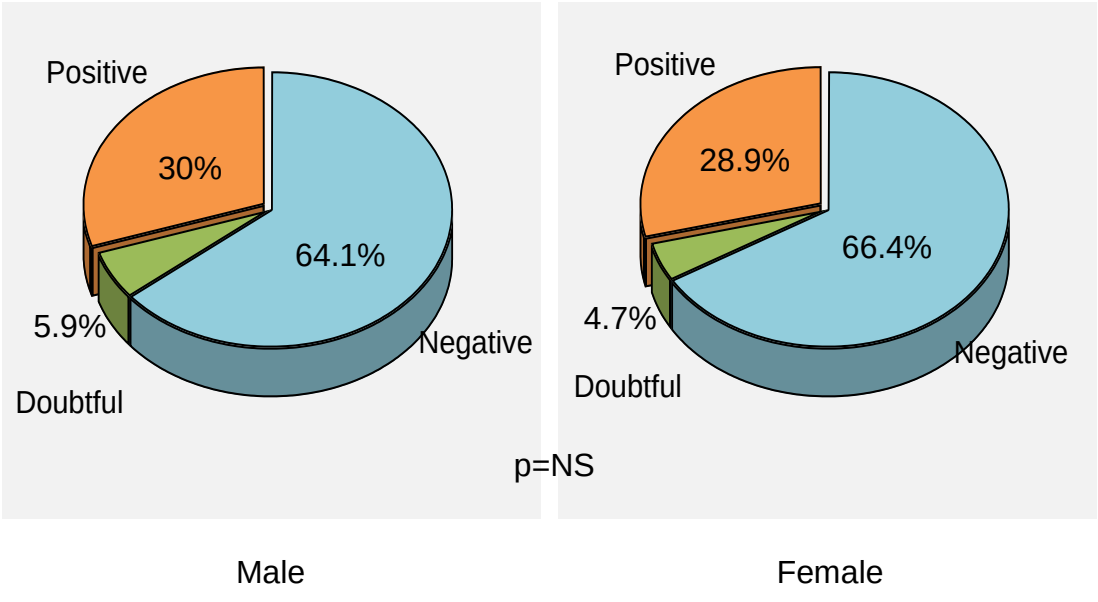
Figure 33: Anti-Toxoplasma IgG titers by age groups



6.3.2.2. Seroprevalence of toxoplasmosis according to gender

There was no difference in prevalence by gender. Females had positive IgG antibodies in 29% and males in 30% (Figure 34).

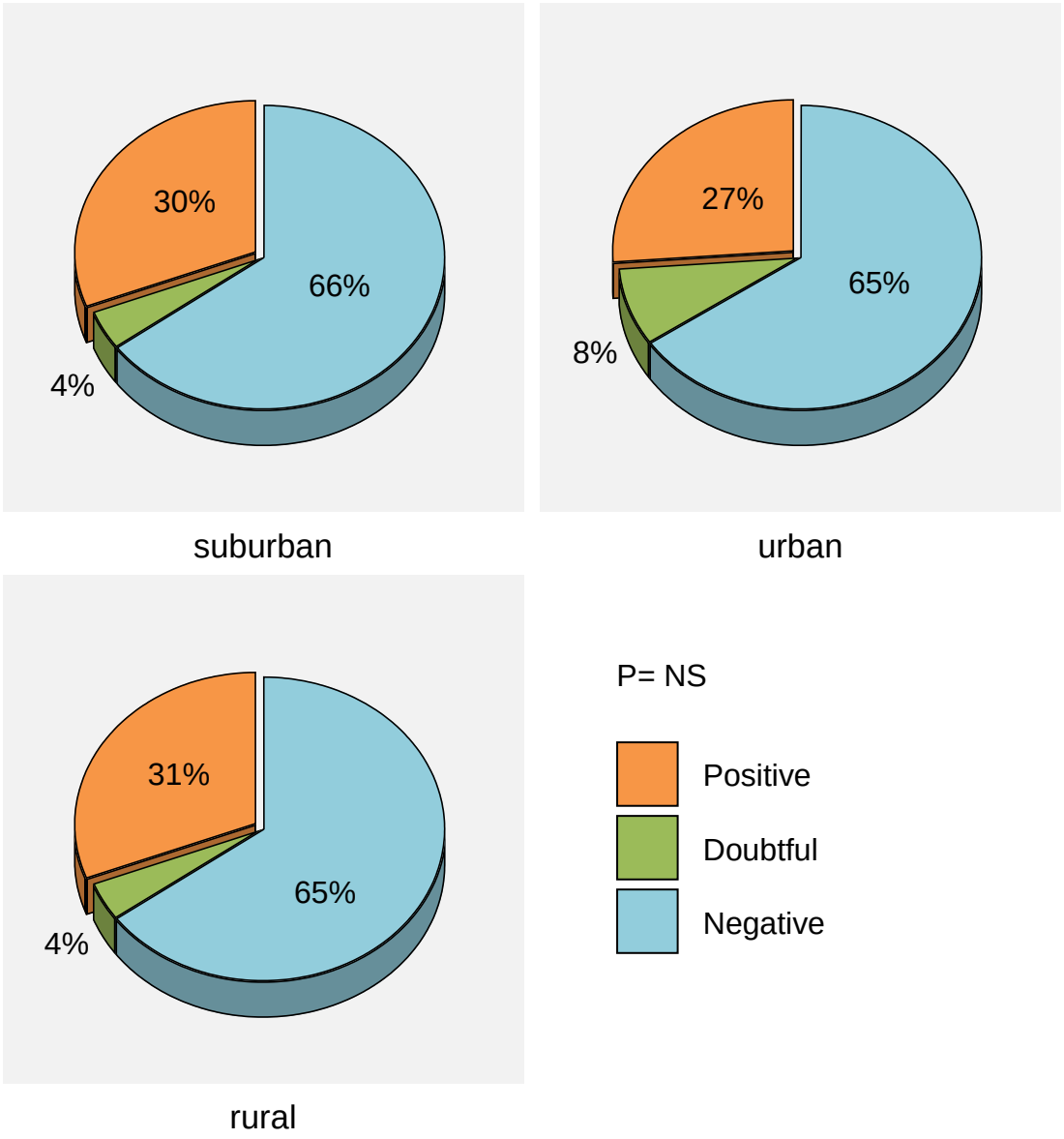
Figure 34: Anti-Toxoplasma IgG antibodies by gender



6.3.2.3. Seroprevalence of toxoplasmosis according to residence

In our study the proportion of children living in rural areas was slightly higher than those living in urban areas. However, there was no a statistically significant difference in prevalence of *Toxoplasma gondii* infection in relation to residential areas. Figure 35 shows anti-*Toxoplasma gondii* IgG titers by place or residence.

Figure 35: Anti-Toxoplasma IgG antibodies by residence



6.3.2.4. Lifestyle risk factors

Environmental exposure was analysed, including close contact with cats and soil, consumption of raw meat and vegetables, drinking untreated water or milk and personal hygiene habits (hands washing).

Table 13: Lifestyle risk factors of *Toxoplasma gondii* infection I

Variable	Sero-negativity N (%)	Sero-positivity N (%)	P- value
Eating raw meat			
Yes	18(39.1%)	28(60.9%)	<0.001
No	176(74.6%)	60(25.4%)	
Eating raw vegetables			
Yes	20(38.5%)	32(61.5 %)	<0.001
No	174(75.7%)	56(24.3%)	
Source of drinking water			
Public water source	111(63.1%)	65(36.9%)	<0,01
Alternative water source	83(78.3%)	23(21.7%)	
Drinking milk			
Unpasteurized	31(61%)	20(39%)	NS
Pasteurized	179(73%)	67(27%)	
Personal hygiene			
Yes	133(70.4%)	56(29.6 %)	N.S
No	61(65.6%)	32(34.4%)	
Contact with soil			
Yes	53(49.5%)	54(50.5%)	<0.001
No	141(80.6%)	34(19.4%)	

Percentage by rows
Raw meat: frequent eating of raw or undercooked meat or homemade dried meat.
Alternative water: drinking water from private wells or rain water deposits.
Raw vegetables: frequent eating raw vegetables and fruits without disinfestation.
Soil contact: frequent contact with soil without proper hands cleaning.

Table 14: Lifestyle risk factors factors of *Toxoplasma gondii* infection II

Variable	Sero-negativity N (%)	Sero-positivity N (%)	P- value
Contact with cats			
Yes	33(43.4%)	43(56.6%)	<0.001
No	161(78.2%)	45(21.8%)	
Contact with house cats			
Yes	16(43.2%)	21(56.8%)	<0.001
No	178(72.7%)	67(27.3%)	
Neighbourhood cats			
Yes	20(36.4%)	35(63.6%)	<0.001
No	173(76.5%)	63(32.5%)	
More than two house cats			
Yes	10(62.5%)	6(37.5%)	N.S
No	148(69.2%)	82(30.8%)	
Contact with dogs			
Yes	17(68 %)	8(32%)	N.S
No	177(68.9%)	88(31.1%)	

Percentage by rows

There were significant relations of *Toxoplasma gondii* infection with eating raw meat, eating raw vegetables and frequent contact with cats (Table 13 and Table 14). Drinking water from alternative water sources (private wells and rain deposits), although being generally untreated water sources, paradoxically showed some protective effect against *T. gondii* infection.

6.3.2.5. Blood transfusions

Previous blood transfusions were not related to *Toxoplasma gondii* infection (Table 15).

Table 15: Blood transfusions and Toxoplasma gondii infection

Blood transfusions	Sero-negativity N (%)	Sero-positivity N (%)	P- value
Yes	10(83.3%)	2(16.7%)	N.S
No	148(68.1%)	86(31.9%)	

Percentage by rows

6.3.2.6. Multivariate analysis of risk factors for *Toxoplasma gondii* infection

Independent relations of demographical and environmental variables with infection with *Toxoplasma gondii* were explored by stepwise logistic regression. Table 16 shows independent risk factors for *Toxoplasma gondii* infection.

Table 16: Risk factors for *Toxoplasma gondii* infection in study population (logistic regression coefficients)

				OR 95% C.I.	
	ß coefficient	p	Odds ratio	Lower limit	Upper limit
Raw meat	1.212	0.002	3.36	1.56	7.25
Alternative water	-1.002	0.004	0.37	0.19	0.72
Raw vegetables	1.408	0.000	4.09	1.87	8.93
Soil contact	1.035	0.002	2.82	1.5	5.35
Neighbourhood cats	1.756	0.000	5.79	2.82	11.91

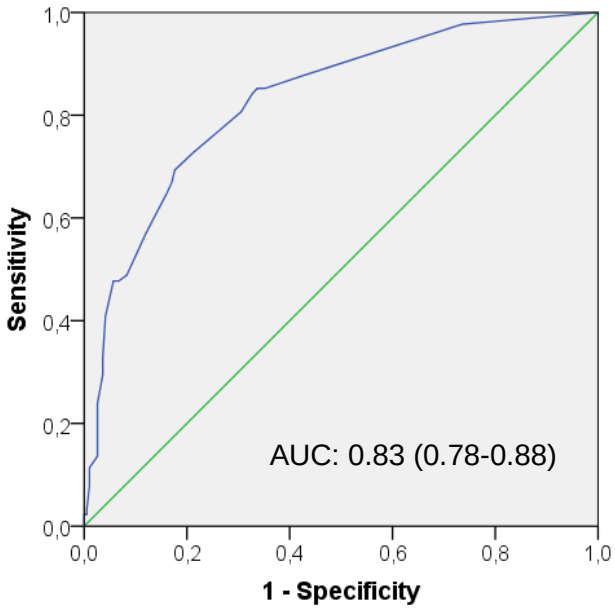
Raw meat: frequent eating raw or undercooked meat or homemade dried meat.
Alternative water: drinking water from private wells or rain water deposits.
Raw vegetables: frequent eating raw vegetables and fruits without disinfestation.
Soil contact: frequent contact with soil without proper hands cleaning.
Neighbourhood cats: presence of cats in neighbours (includes home cats).

Stepwise method was conditional backward selection. Cases with doubtful IgG titers were excluded. Table 18 shows independent risk factors for *Toxoplasma gondii* infection.

Variables not included in model (not independently related with *T. gondii* seropositivity) were sex, age and age group, residence, drinking unpasteurized milk, blood transfusions, personal hygiene habits, contact with cats, and contact with dogs.

Model Cox R^2: 0.268; Nagelkerke R^2: 0.378. Model classified correctly 79% of cases. Goodness of fit was evaluated with ROC curve analysis of predicted values (Figure 36). Area under the curve was 0.83 (95% confidence limits: 0.78-0.88).

Figure 36: Risk factors for Toxoplasma infection. ROC curve of LR predicted values.



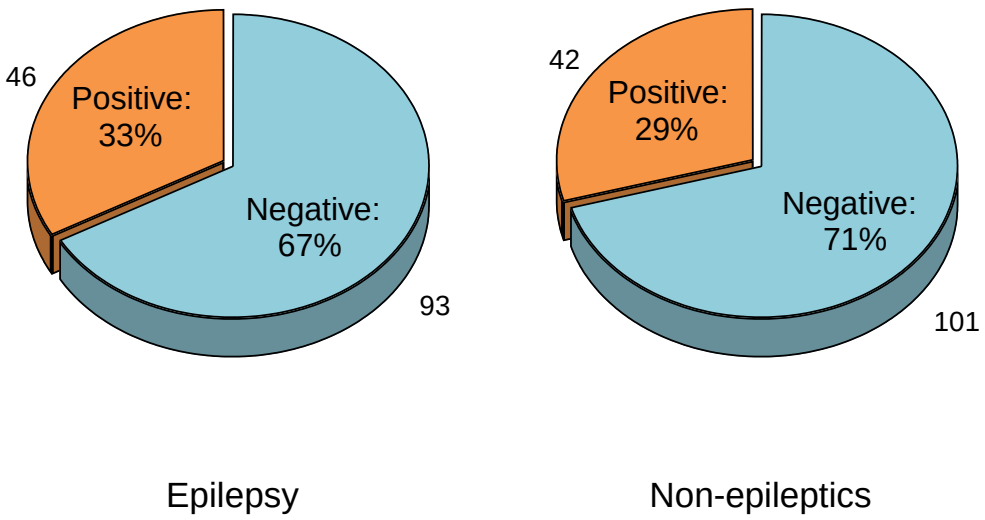
6.3.3. Relations of toxoplasmosis and epilepsy

6.3.3.1. Comparing anti-*Toxoplasma* IgG between epileptic patients and non-epileptic controls

6.3.3.1.1. Prevalence of *T. gondii* infection

There were not differences in the prevalence of *Toxoplasma gondii* infection between epileptic children and non-epileptic controls (33,1% and 29,4% respectively). Cases with doubtful titers were excluded. (Figure 37)

Figure 37: Prevalence of toxoplasma gondii infection in epileptic children and not-epileptic controls

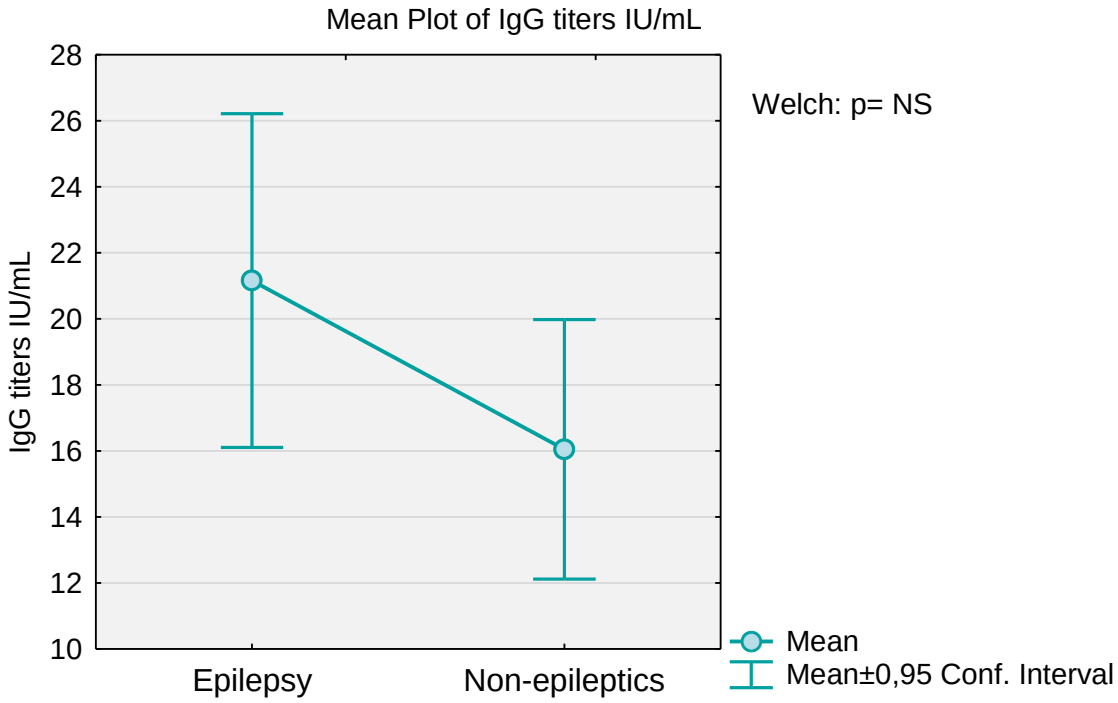


Subjects with doubtful IgG titers (8-12 IU/mL) were excluded.

6.3.3.1.2. Antibody titers against *T. gondii*

Figure 38 shows titers of IgG anti-*T. gondii* in epileptic patients and non-epileptic controls. There were not significant differences.

Figure 38: Anti-Toxoplasma IgG titers in epileptic children and non-epileptic contols

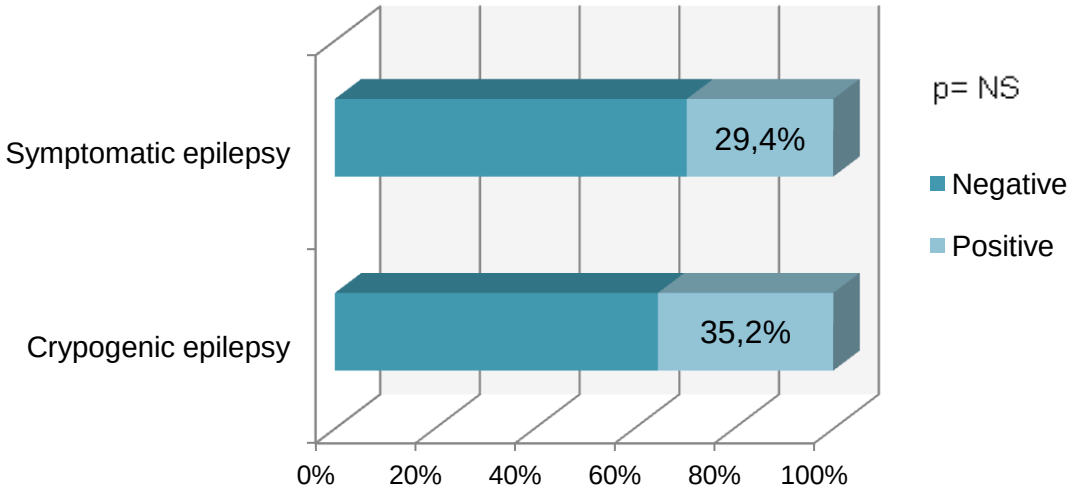


6.3.3.2. Comparing anti-*Toxoplasma* IgG within epileptic patients

6.3.3.2.1. Prevalence of infection

Figure 37 shows prevalence of *Toxoplasma gondii* infection in patients with cryptogenic epilepsy and patients with symptomatic epilepsy. Patients with doubtful titers (8-12 IU/mL) were excluded. Differences were not significant.

Figure 39: Anti-Toxoplasma IgG prevalence in epileptic patients.



Patients with doubtful IgG titers (8-12 IU/mL) were excluded

6.3.3.2.2. Titers of anti-Toxoplasma IgG

Figure 40 shows titers of anti-*Toxoplasma gondii* IgG in patients with cryptogenic epilepsy and in patients with symptomatic epilepsy. Mean IgG titers were higher in patients with cryptogenic epilepsy (Cryptogenic epilepsy 24.7±7.6 IU/mL; Symptomatic epilepsy 15.3 ±5.8 IU/mL; Welch's $p<0.05$). As maximum values detected were limited to 101 IU/mL this difference cannot be due to outliers' effect.

Percentage of patients with anti-toxoplasma IgG titers over 75 UI/mL was higher in cryptogenic epilepsy patients (Figure 41).

Figure 40: Anti-Toxoplasma IgG titers in epileptic patients.

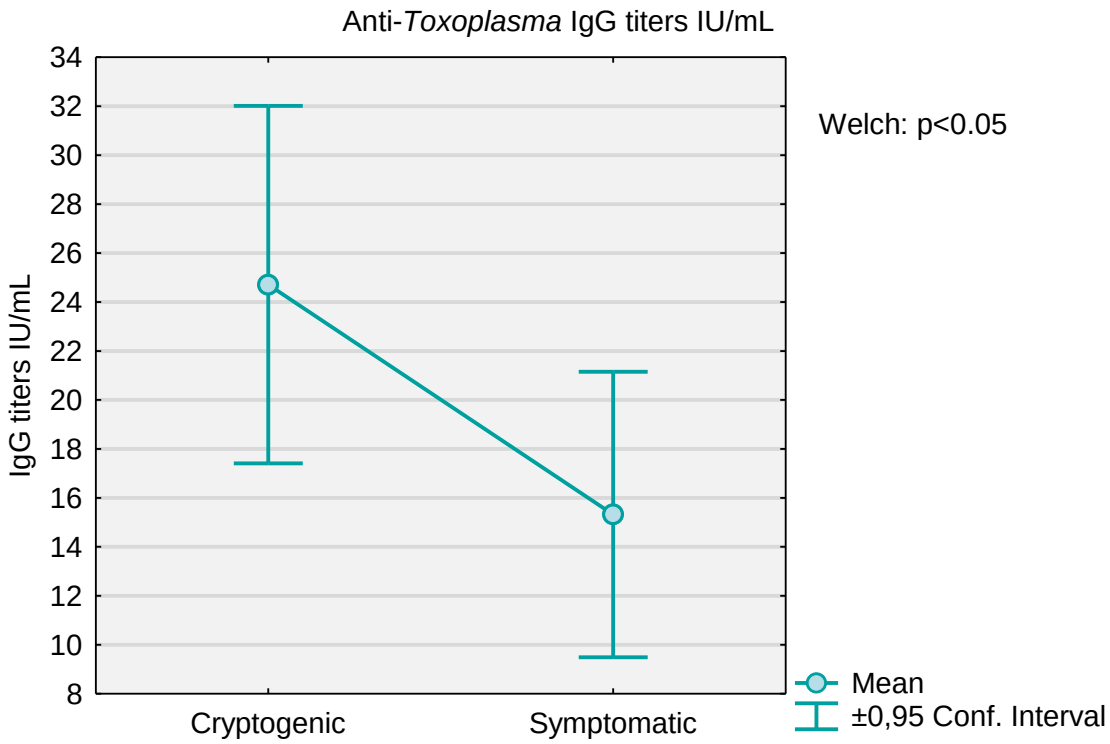
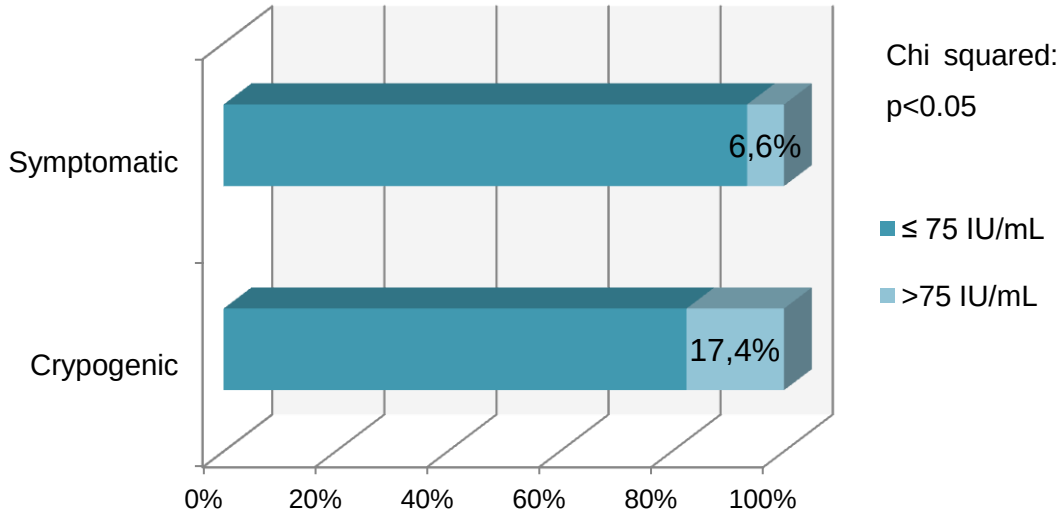


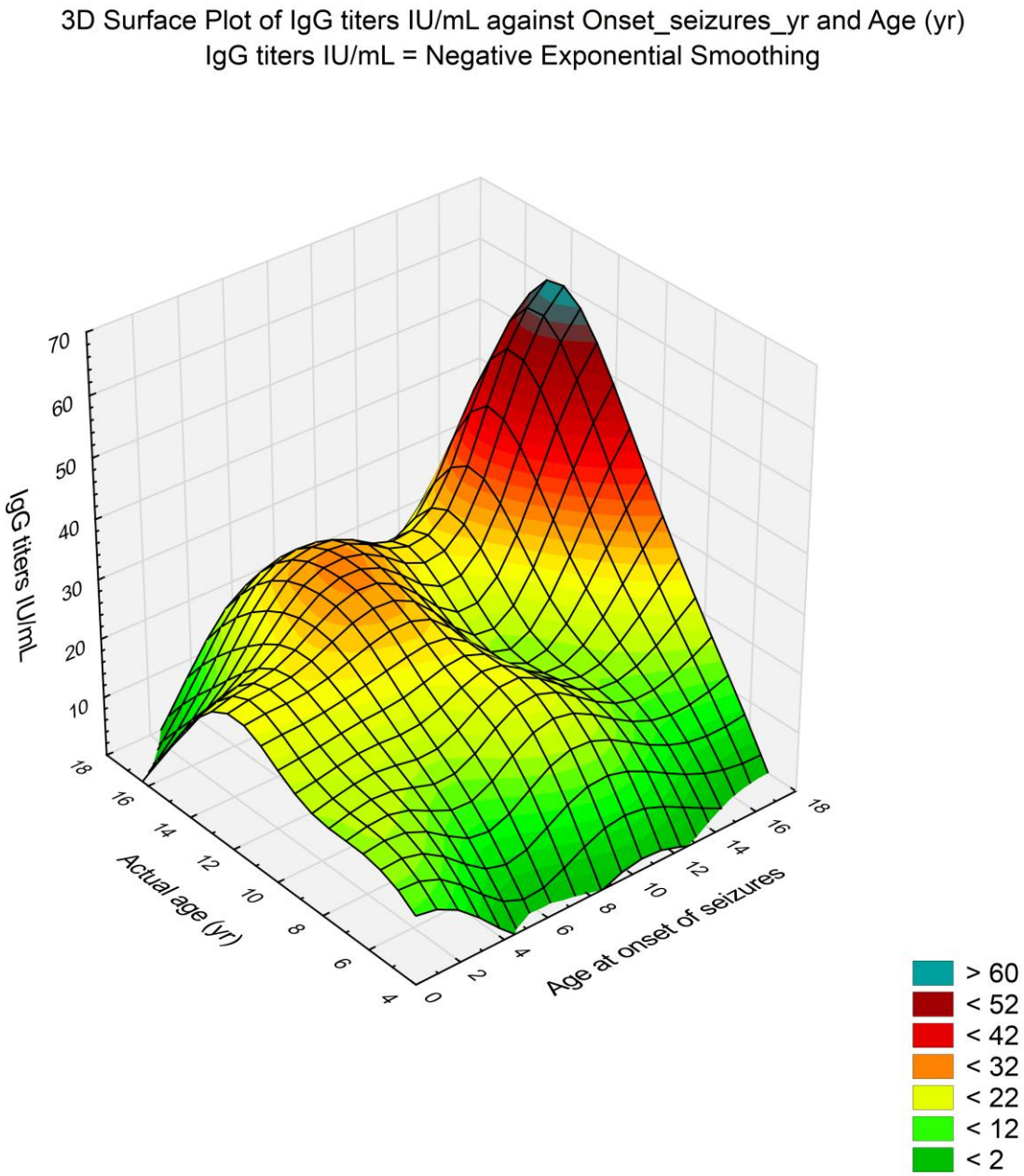
Figure 41: High anti-Toxoplasma IgG titers in epileptic patients.



Patients with doubtful IgG titers (8-12 IU/mL) were excluded.

Figure 42 shows exploratory plot of relationships between IgG titers with age and age at epilepsy onset. It shows some association between the age over 12 years and higher IgG titres that was confirmed posteriorly by logistic regression analysis.

Figure 42: Relations of IgG titers with age and age at epilepsy onset.

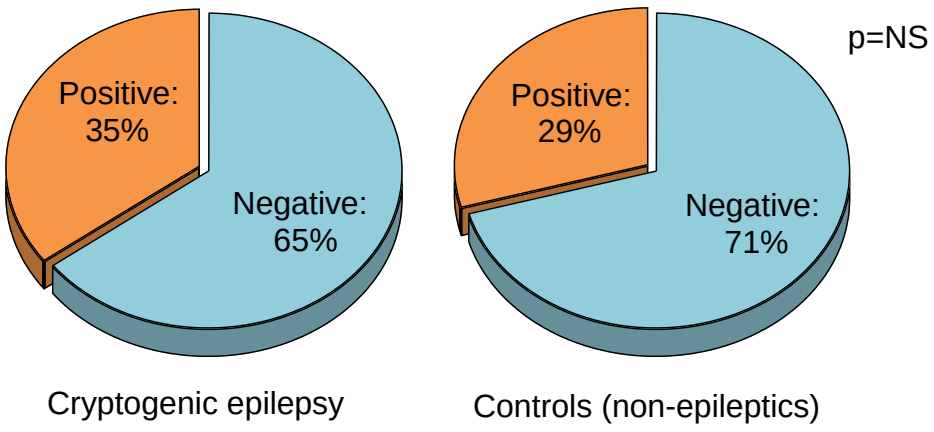


6.3.3.3. Comparing toxoplasma infection between patients with cryptogenic epilepsy and controls without epilepsy

6.3.3.3.1. Prevalence of Toxoplasma gondii infection

There were no significant differences in prevalence of *Toxoplasmosis* between overall group of patients with cryptogenic epilepsy and non-epileptic controls (Figure 43).

Figure 43: Prevalence of toxoplasmosis in patients with cryptogenic epilepsy and in non-epileptic controls.



Patients with doubtful IgG titers (8-12 IU/mL) were excluded

6.3.3.3.2. Anti-Toxoplasma IgG titers

Figure 44 shows mean anti-toxoplasma IgG titers of children with cryptogenic epilepsy and non-epileptic control. Patients with cryptogenic epilepsy had higher mean anti-toxoplasma IgG titers than controls (cryptogenic epilepsy 24.7±8.4 IU/mL; controls 16.1±4 IU/mL). This is due to increased rate of patients with high titers of anti-Toxoplasma IgG in patients with cryptogenic epilepsy (Figures 45 and 46).

Figure 44: Anti-Toxoplasma IgG titers in patients with cryptogenic epilepsy and in non- epileptic controls.

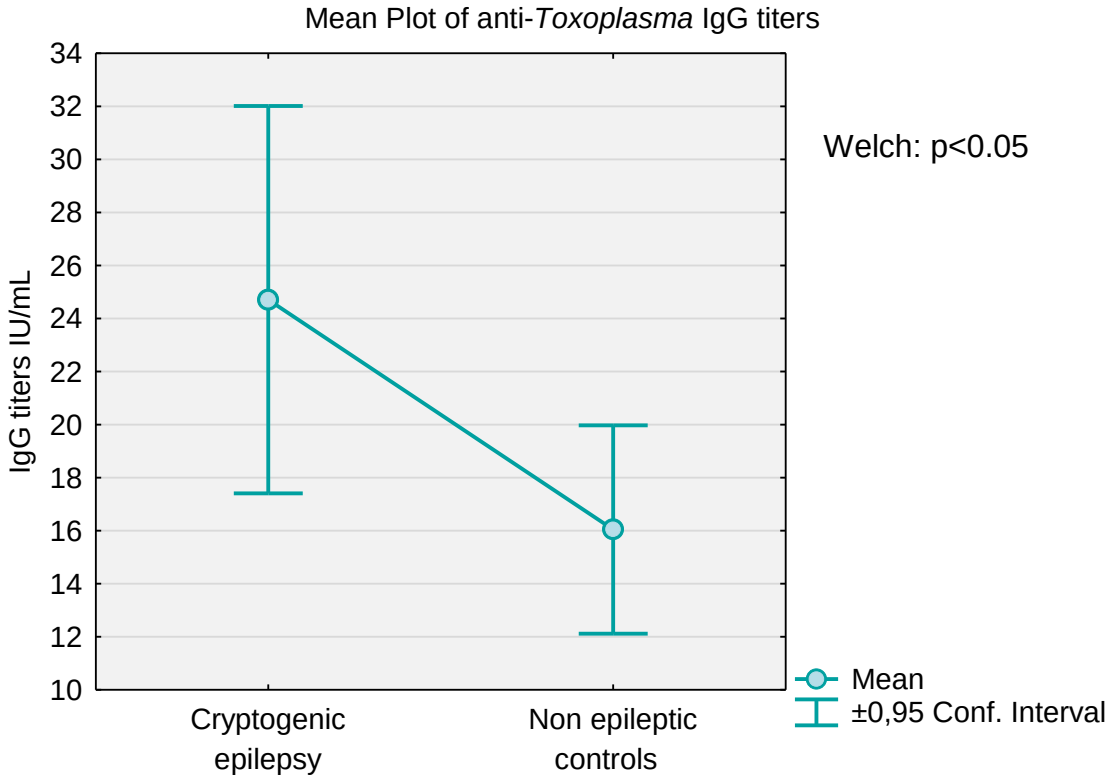
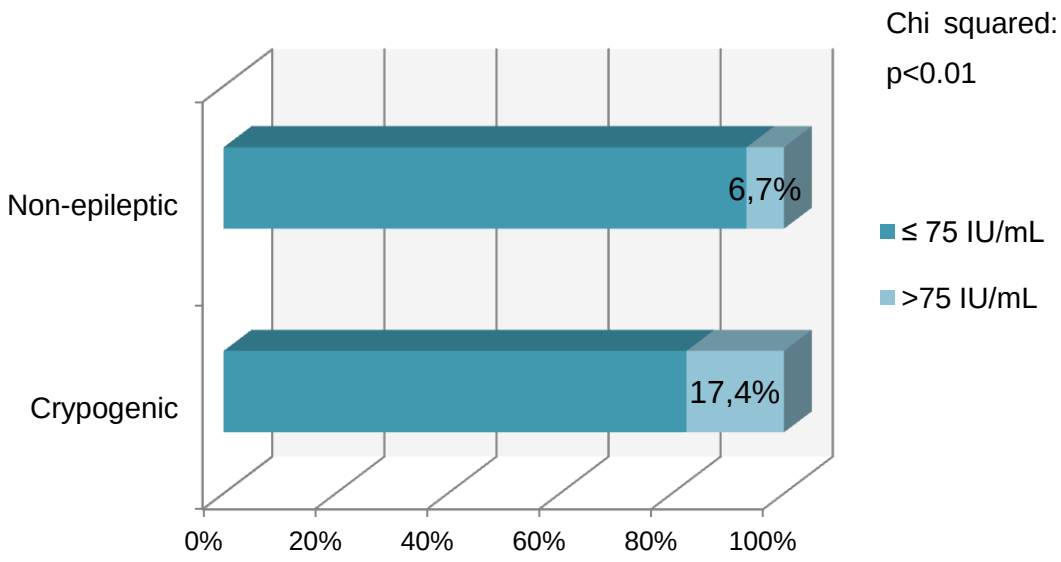
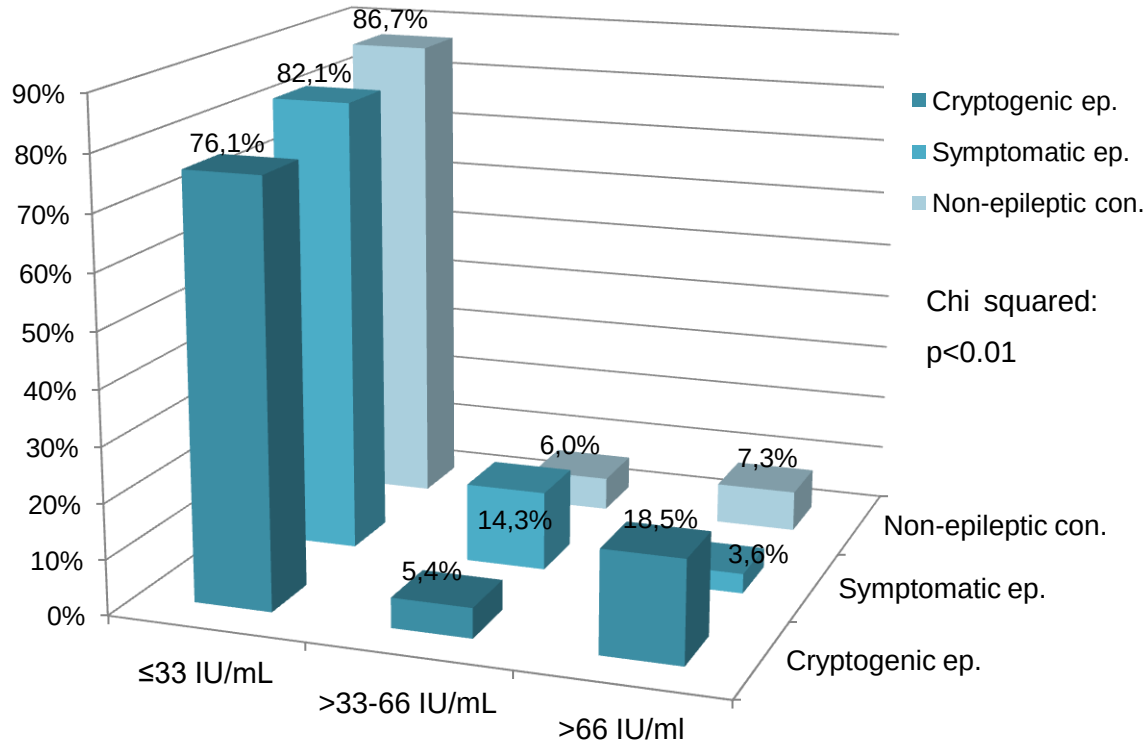


Figure 45: Prevalence of high titers of anti-Toxoplasma IgG in patients with cryptogenic epilepsy and non-epileptic controls.



Patients with doubtful IgG titers (8-12 IU/mL) were excluded.

Figure 46: Titers of anti-Toxoplasma IgG



6.4. Epilepsy

This section resumes characteristics of epilepsy in studied patients. These patients are not representative of population of epileptic children in Libya. They were selected at only one treatment center and according to the objectives of this study.

6.4.1. Type of seizures

Types of seizures were grouped in main categories according to 2016 classification of ILAE. Classification shown is not exhaustive.

There were differences in types of seizures between suspected cryptogenic epilepsy patients and patients with secondary epilepsy (Table 17, Figure 47 and 48). Absence seizures were present only in suspected cryptogenic epilepsy.

Table 17: Type of seizures (% in columns).

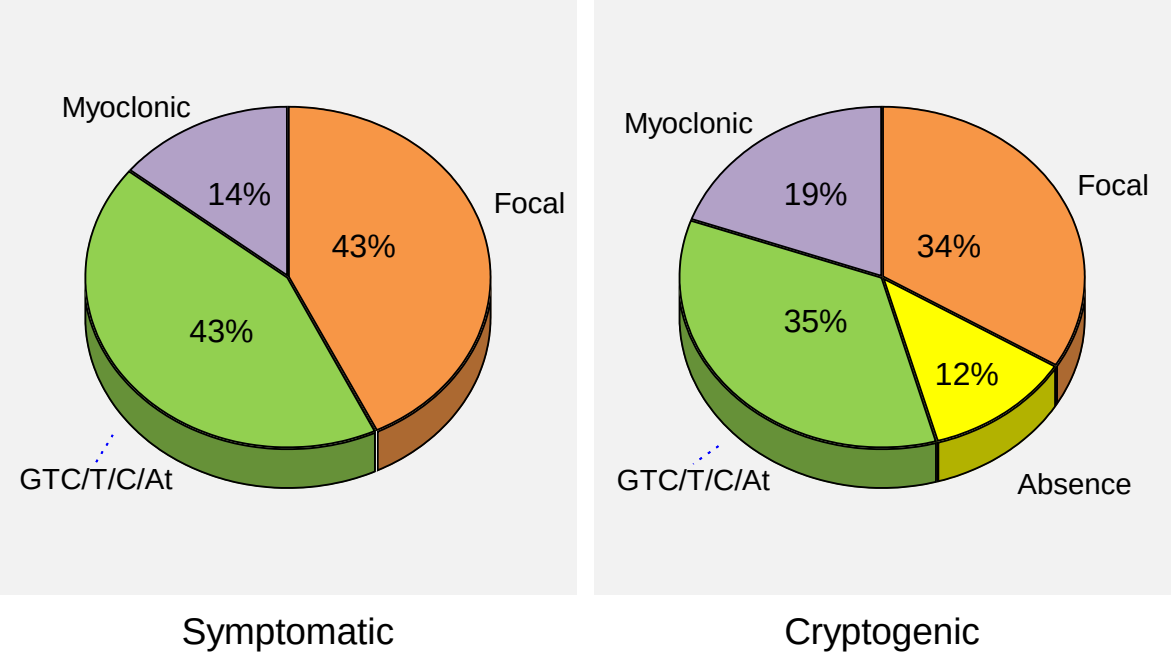
Type of seizures	Cryptogenic	Symptomatic	Total
Focal	31 (33.7%)	24 (42.9%)	55 (37.2%)
Generalized:			
- Absence	11 (12%)	0 (0%)	11 (7.4%)
- Motor:			
TC+T+C+At	32 (34.8%)	24 (42,9%)	56 (37.8%)
Myoclonic	18 (19.6%)	8 (14.3%)	26 (17.6%)
Total	92 (100%)	56 (100%)	148 (100%)

Percentage by columns

Predominant seizures. TC: tonic clonic; T: tonic; C: clonic; At: atonic.

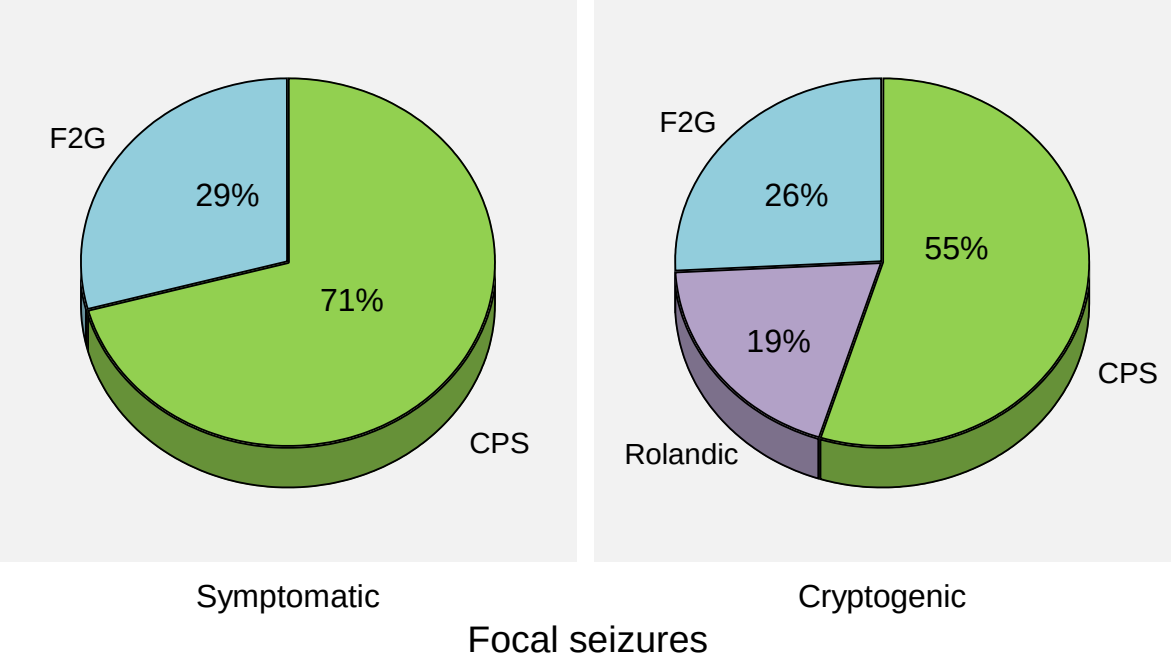
Cryptogenic vs. symptomatic: Chi-squared, $p<0.05$

Figure 47: Type of seizures.



GTC: generalized tonic clonic; T: tonic; C: clonic; At: atonic

Figure 48: Types of focal seizures.



F2S: focal secondarily generalized; CPS: complex partial seizures

6.4.2. Control of seizures

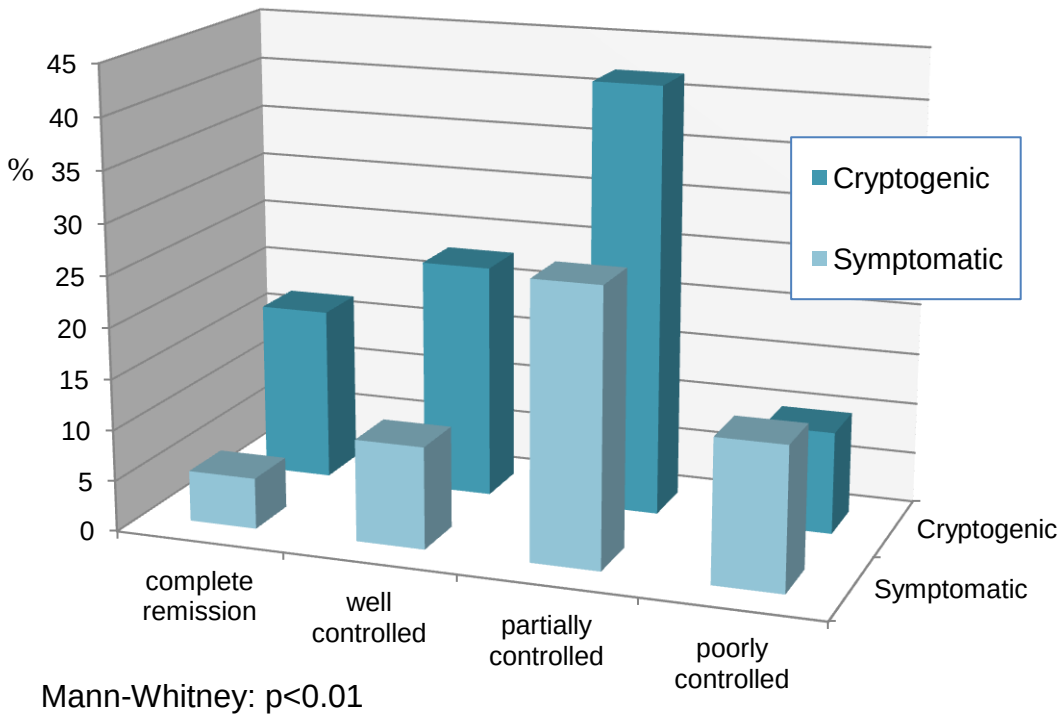
Figure 49 shows degree of seizure control in epileptic patients. Seizures' control was categorized in four groups:

- Complete remission: absence of seizures in 2 years.
- Well controlled: absence of seizures in 6 months.
- Partially controlled: some seizures in 6 months.
- Poorly controlled: weekly or daily seizures.

Patients with cryptogenic epilepsy had a better control of seizures than patients with secondary epilepsy, with less than 10% of poorly controlled seizures (Figure 47).

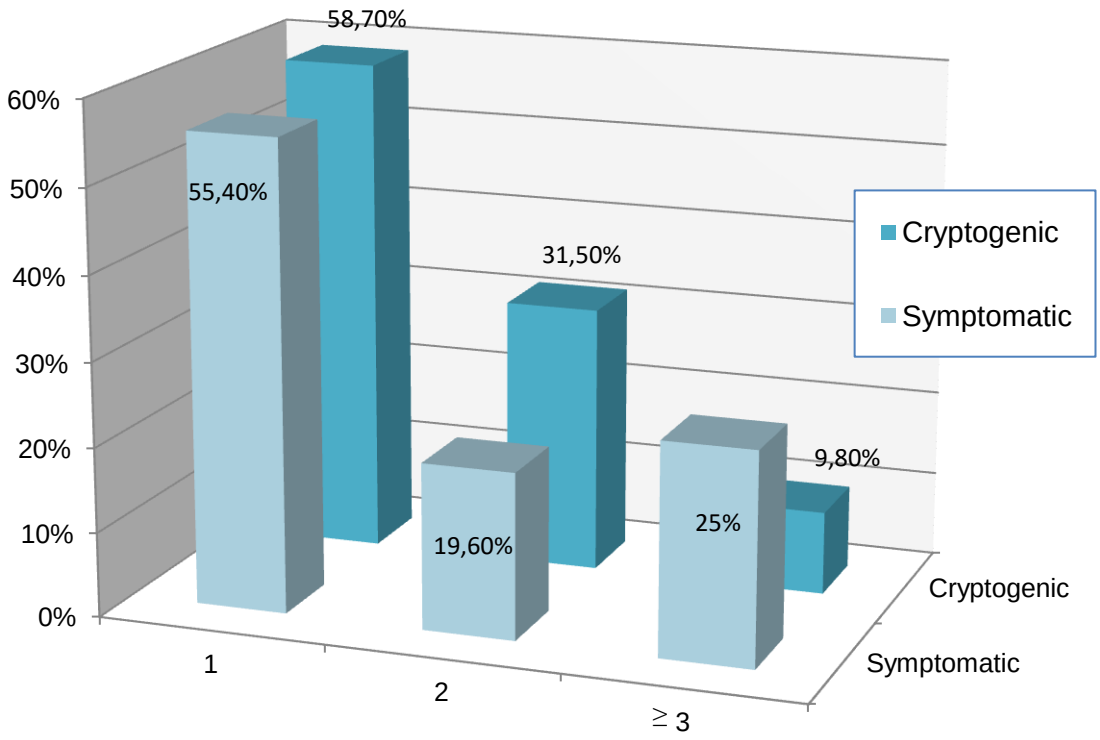
Figure 48 shows need of combination therapy in children with cryptogenic epilepsy and in symptomatic epilepsy. Patients with symptomatic epilepsy had a higher percentage of children needing 3 drugs.

Figure 49: Degree of seizures control in epileptic patients.



Mann-Whitney: p<0.01

Figure 50: Number of antiepileptic drugs required in patients with cryptogenic and symptomatic epilepsy.



Chi squared: $p<0.05$

6.4.3. Toxoplasmosis and control of seizures:

Possible relation of infection by *Toxoplasma gondi*i and control of seizures was explored. Table 18 and Table 19 show relation of toxoplasmosis and control of seizures and number of antiepileptic drugs. No relation was found between these variables and toxoplasma infection.

Table 18: Toxoplasma infection and control of epilepsy

Anti-Toxoplasma ab.	Control of epilepsy			
	Complete remission	Well controlled	Partially controlled	Poorly controlled
Negative	15 (16.1%)	18 (19.4%)	46 (49.5)	14 (15.1)
Positive	6 (13%)	12 (26.1%)	20 (43.5%)	8 (17.4%)

Mann-Whitney: p=NS

Table 19: Toxoplasmosis and number of antiepileptic drugs

Anti-Toxoplasma ab.	Nº antiepileptic drugs		
	1	2	≥3
Negative	53 (57%)	27 (29%)	13 (14%)
Positive	27 (58.7%)	11 (23.9%)	8 (17.4%)

Mann-Whitney: p=NS

6.4.4. Toxoplasmosis seroprevelance and type of seizures

Table 20 and 21 shows relations between the type of epileptic seizures and prevalence of *Toxoplasma gondii* infection. Patients with focal seizures had a higher rate of infection by *Toxoplasma gondii* (Figure 51 and 52). Figure 53 shows a more detailed distribution of seizure types and its relation with toxoplasma seropositivity. Patients with doubtful anti-*Toxoplasma* IgG titers were excluded.

Table 20: Toxoplasmosis seroprevelance and type of seizures I

Type of seizures	Positive	Negative	Chi-squared p value
Generalized	22 (29%)	66 (71%)	<0.05
Focal	24 (47.1%)	27 (52.9%)	

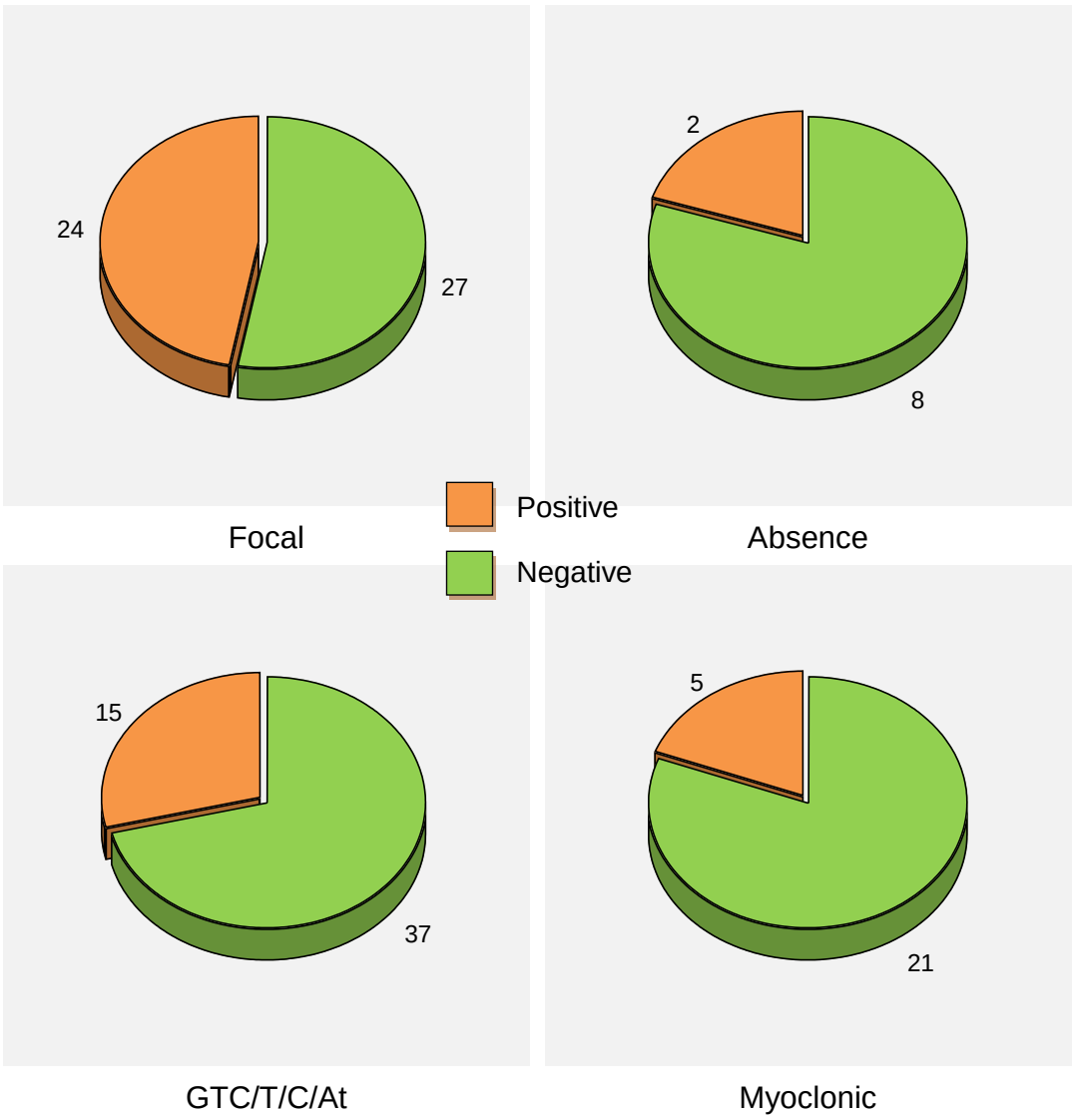
Percentage by row

Table 21: Toxoplasmosis seroprevelance and type of seizures II

		Type of seizures				
Anti-Toxoplasma ab. result		A	GTC	M	F	Total
Negative	Count	8	37	21	27	93
	% within column	80 %	71.2 %	80.8 %	52.9 %	66.9 %
Positive	Count	2	15	5	24	46
	% within column	20 %	28.8 %	19.2 %	47.1 %	33.1 %
Total	Count	10	52	26	51	139
	% within column	100%	100%	100 %	100 %	100%

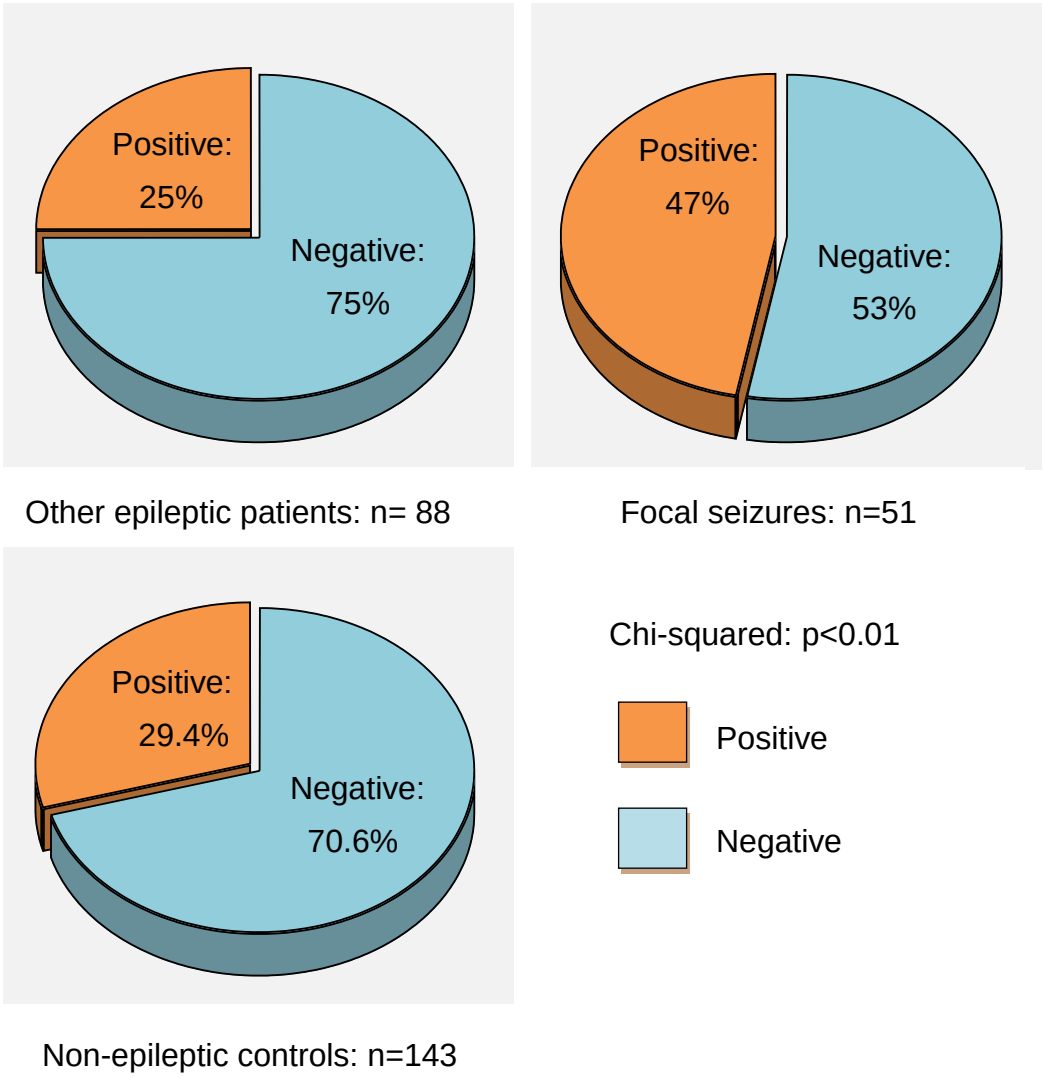
A: absence seizures. GTC: generalized, tonic clonic, tonic, clonic and atonic seizures. M: myoclonic seizures. F: focal seizures.

Figure 51: Relation of anti-Toxoplasma IgG in epileptic patients to type of seizures.



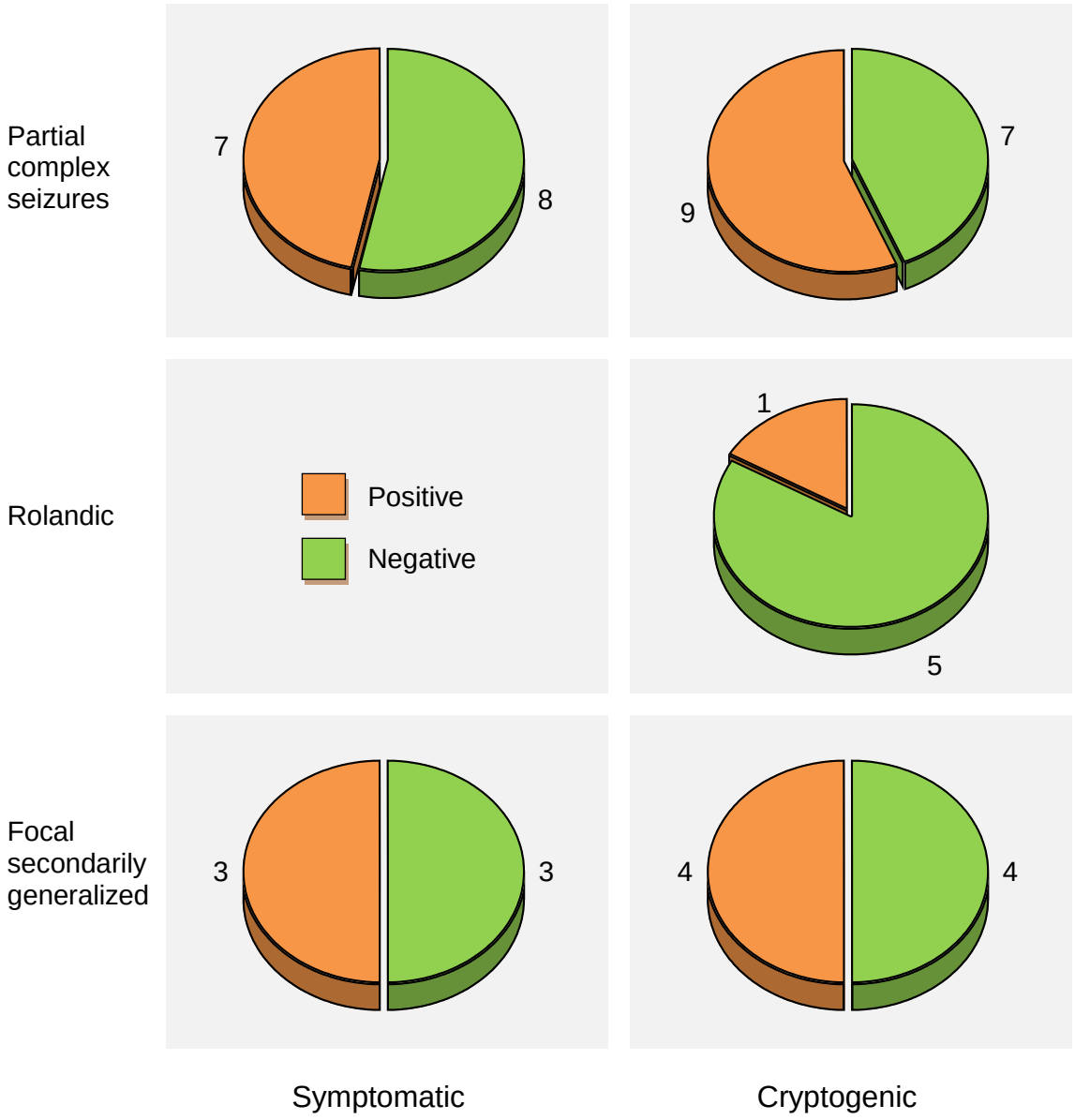
Patients with doubtful IgG titers (8-12 IU/mL) were excluded

Figure 52: Relation of anti-Toxoplasma IgG positivity to type of seizures.



Subjects with doubtful IgG titers (8-12 IU/mL) were excluded.

Figure 53: Prevalence of Toxoplasma gondii infection in epileptic patients with focal seizures.

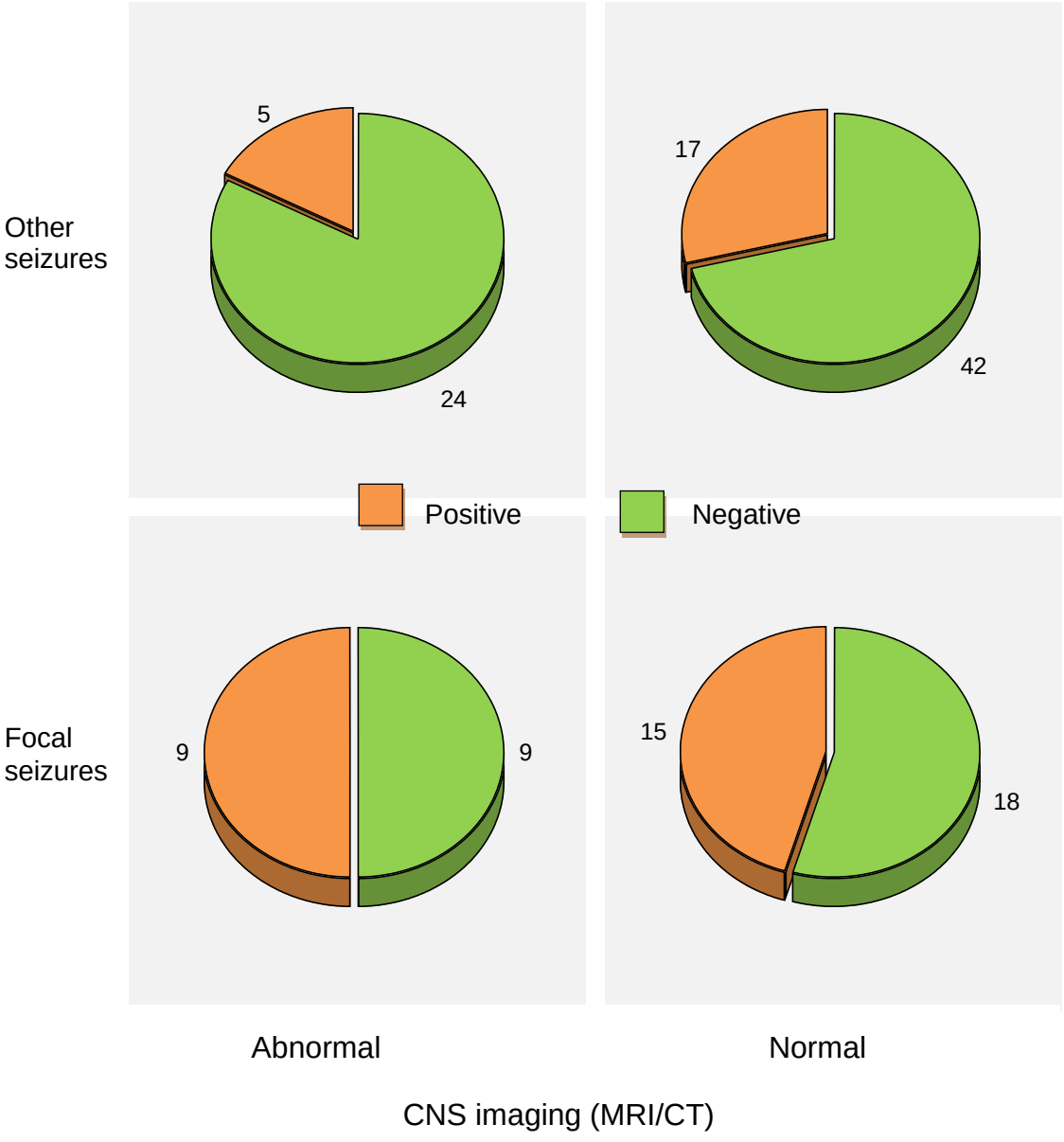


Subjects with doubtful IgG titers (8-12 IU/mL) were excluded.

6.4.4.1. Prevalence of toxoplasma infection by type of seizure and CNS imaging results

There were no significant differences in prevalence of Toxoplasma gondii infection between epileptic patients with normal or abnormal CNS imaging. Figure 54 shows frequency of TG infection by seizure type and results of CNS imaging.

Figure 54: Prevalence of *Toxoplasma gondii* infection in epileptic patients. Combined effect of type of seizures and results of CNS imaging.



Subjects with doubtful IgG titers (8-12 IU/mL) were excluded.

Table 22 resumes some characteristics of patients with focal seizures and abnormal MRI. Periventricular leukomalacia was the abnormality diagnosed in most of these patients.

Table 22: Patients with focal epilepsy and abnormal CNS imaging

Age (yr)	Epilepsy onset (yr)	Brain MRI	Speech disability	Toxoplasma IgG
9,7	2,5	PVL	Mild	Negative
12,6	1,1	PVL II	Mild	Negative
12	8,9	PVL	Mild	Negative
14,3	2	PVL	Normal speech	Negative
13,5	4	PVL + SCL	Severe	Negative
11,4	4	Miscellaneous	Severe	Negative
8,3	2	Basal ganglia	Speechless	Negative
15	4	PVL + SCL	Severe	Negative
6,7	1	IVHg III	Severe	Negative
11,4	4	IVHg II	Mild	Positive
11,9	2,5	PVL I	Normal speech	Positive
14	6	IVHg	Mild	Positive
14	5	PVL	Normal speech	Positive
6,5	1	PVL	Normal speech	Positive
8	8	Infarction	Mild	Positive
6	2	PVL	Severe	Positive
6,2	0,9	PVL + SCL	Speechless	Positive
12,6	5	BIL PVL	Severe	Positive

PVL: periventricular leukomalacia; SCL: subcortical lesions; IVHg: intraventricular haemorrhage; BIL: bilirrubin encephalopaty.

6.4.5. Multivariate analysis of relationships of epilepsy with *toxoplasma gondii* infection

6.4.5.1. Variables related to *Toxoplasma gondii* infection in all subjects studied

Logistic regression analysis was utilized to identify variables independently related to *Toxoplasma gondii* infection. Dependent variable was positivity or negativity of anti-*Toxoplasma* IgG. Patients with doubtful titers were excluded. Selection method was backward stepwise exclusion.

Five dummy variables were created as follows and included in all regression analysis:

1. Symptomatic epilepsy: Yes=1, No=0.
2. Cryptogenic epilepsy: Yes=1, No=0.
3. Non-epileptic controls: Yes=1, No=0.
4. Focal seizures: Yes=1, No=0.
5. Other seizures: Yes=1, No=0.

6.4.5.1.1. Variables related to Toxoplasma gondii infection, without considering behavioral risk factors

Other variables included in this analysis were age group, sex, speech disability and residence.

Logistic regression results:

The only variable selected in logistic regression equation was "Focal seizures", which was positively related to *Toxoplasma gondii* infection (Table 23). Model Cox R^2: 0.024; Nagelkerke R^2: 0.034.

Table 23: Factors related to Toxoplasma gondii infection (logistic regression coefficients)

				OR 95% C.I.	
	ß coefficient	p	Odds ratio	Lower limit	Upper limit
Focal seizures	0.84	0.008	2.32	1.25	4.32

6.4.5.1.2. Variables related to *Toxoplasma gondii* infection, adjusted by behavioral risk factors

Other variables included in this analysis were age group, sex, speech disability, residence, eating raw meat, eating raw vegetables, alternative source of drinking water, drinking unpasteurized milk, contact with soil, personal hygiene, contact with cats, and neighbourhood cats.

Logistic regression results:

Table 24 shows regression analysis results. After adjustment by behavioural risk factors, "Focal seizures" continues to be independently related to *Toxoplasma gondii* infection.

Table 24: Factors related to Toxoplasma gondii infection, including behavioral risk factors (logistic regression coefficients)

				(Exp ß) 95% C.I.	
	ß coefficient	p	Odds ratio (Exp ß)	Lower limit	Upper limit
Focal seizures	1.011	0.010	2.75	1.27	5.94
Raw meat	1.151	0.004	3.16	1.44	6.94
Alternative water	-1.028	0.003	0.36	0.18	0.71
Raw vegetables	1.566	0.000	4.79	2.12	5.16
Soil contact	0.985	0.003	2.68	1.39	5.16
Neighbourhood cats	1.779	0.000	5.92	2.85	12.32

Raw meat: frequent eating raw or undercooked meat or homemade dried meat.
Alternative water: drinking water from private wells or rain water deposits.
Raw vegetables: frequent eating raw vegetables and fruits without disinfestation.
Soil contact: frequent contact with soil without proper hands cleaning.
Neighbourhood cats: presence of cats in neighbours.

Model Cox R^2: 0.285; Nagelkerke R^2: 0.401. Model classified correctly 78.9 % of cases.

6.4.5.2. Variables related to *Toxoplasma gondii* infection in epileptic patients

6.4.5.2.1. Without behavioral risk factors:

In this analysis, non-epileptic controls were excluded. The dependent variable was positivity of anti-*Toxoplasma* IgG. Patients with doubtful titers were excluded.

For analysing the effect of the type of epilepsy, four dummy variables were created and coded as follows:

1. Focal seizures: Yes=1, No=0.
2. Generalized absence seizures: Yes=1, No=0.
3. Generalized myoclonic seizures: Yes=1, No=0.
4. Generalized tonic clonic, tonic, clonic or atonic seizures: Yes=1, No=0.

Degree of epilepsy control was coded in three dummy variables:

1. Remission of seizures: Yes=1, No=0.
2. Not well controlled: Yes=1, No=0.
3. Poorly controlled: Yes=1, No=0.

Other variables included in this analysis were age, age group, sex, age at epilepsy onset, number of antiepileptic drugs. Variable selection method was backward stepwise exclusion.

Logistic regression results:

Only "Focal seizures" was independently related to *Toxoplasma gondii* infection (Table 25). Characteristics of model were: Cox R^2: 0.049; Nagelkerke R^2: 0.068.

Table 25: Factors related to *Toxoplasma gondii* infection in epileptic children (logistic regression coefficients)

				(Exp ß) 95% C.I.	
	ß coefficient	p	Odds ratio (Exp ß)	Lower limit	Upper limit
Focal seizures	0.981	0.009	2.67	1.28	5.54

6.4.5.2.2. Adjusting for behavioural risk factors.

In this analysis, behavioural risk factors were added to the previous set of variables: eating raw meat, eating raw vegetables, alternative source of drinking water, drinking unpasteurized milk, contact with soil, personal hygiene, contact with cats, and neighbourhood cats.

Logistic regression results:

After adjustment by the effect of behavioural risk factors, "Focal seizures" continues to be independently related to *Toxoplasma gondii* infection. "Absence seizures" and "Remission of seizures" were negatively related to *Toxoplasma gondii* infection. Other variables selected were those previously identified as behavioural risk factors, but it is of interest that eating raw vegetables, contact with soil and presence of neighbourhood cats had higher odds ratios in epileptic children than in the whole group (Table 26).

Table 26: Factors related to Toxoplasma gondii infection in epileptic children, including behavioral risk factors (LR coefficients)

				(Exp ß) 95% C.I.	
	ß coefficient	p	Odds ratio (Exp ß)	Lower limit	Upper limit
Focal seizures	1.167	0.040	3.21	1.05	9.80
Absence seizures	-2.607	0.052	0.07	0.01	1.00
Remission of seizures	-2.334	0.009	0.10	0.02	0.55
Raw meat	1.708	0.019	5.52	1.33	22.88
Alternative water	-1.489	0.012	0.23	0.07	0.72
Raw vegetables	3.028	0.001	20.66	3.70	115.23
Soil contact	2.427	0.000	11.32	3.54	36.18
Neighbourhood cats	2.475	0.000	11.89	3.01	46.96

Raw meat: frequent eating raw or undercooked meat or homemade dried meat.
Alternative water: drinking water from private wells or rain water deposits.
Raw vegetables: frequent eating raw vegetables and fruits without disinfestation.
Soil contact: frequent contact with soil without proper hands cleaning.
Neighbourhood cats: presence of cats in neighbours.

Model characteristics were: Cox R^2: 0.442; Nagelkerke R^2: 0.615. Model classified correctly 84.7% of cases.

6.4.5.3. Variables related to high titers of anti-*Toxoplasma* IgG

Dependent variable was titers of anti-*Toxoplasma* IgG ≥ 75 UI/ml. All cases, including those with doubtful titers, were included. Selection method was backward stepwise exclusion.

Following dummy variables were included:

- Symptomatic epilepsy: Yes=1, No=0.
- Cryptogenic epilepsy: Yes=1, No=0.
- Non-epileptic controls: Yes=1, No=0.
- Focal seizures: Yes=1, No=0.
- Generalized seizures: Yes=1, No=0.

Other variables included in this analysis were age group, sex, speech disability, residence and behavioural risk factors (eating raw meat, eating raw vegetables, alternative source of drinking water, drinking unpasteurized milk, contact with soil, personal hygiene, contact with cats, and neighbourhood cats).

Logistic regression results:

Age group 12-18 years and cryptogenic epilepsy were independently and positively related with high titers of anti-Toxoplasma IgG. Behavioural risk factors had the same "behaviour" as in previous analysis (Table 27).

This predictive model had good properties. Model Cox R^2: 0.447; Nagelkerke R^2: 0.621. Model classified correctly 85% of cases.

Table 27: Factors related to high titers of anti-Toxoplasma IgG, including behavioral risk factors (logistic regression coefficients)

				(Exp ß) 95% C.I.	
	ß coefficient	p	Odds ratio (Exp ß)	Lower limit	Upper limit
Age group 12-18 yr.	1.112	0.027	3.04	1.13	8.15
Cryptogenic epilepsy	1.007	0.037	2.74	1.06	7.04
Raw meat	1.069	0.028	2.91	1.12	7.53
Alternative water	-1.159	0.045	0.31	0.10	0.97
Raw vegetables	1.134	0.029	3.11	1.12	8.60
Soil contact	2.546	0.000	12.76	4.26	38.23
Contact with cats	1.112	0.027	3.04	1.13	8.15

Raw meat: frequent eating raw or undercooked meat or homemade dried meat.
Alternative water: drinking water from private wells or rain water deposits.
Raw vegetables: frequent eating raw vegetables and fruits without disinfestation.
Soil contact: frequent contact with soil without proper hands cleaning.

DISCUSSION

7. DISCUSSION

7.1. Study design

The design of our study in three groups was similar to that of the Yazar and Eraky studies. In both cases, a group of patients with cryptogenic epilepsy was compared with non-epileptic controls and with patients with symptomatic epilepsy (Yazar et al. 2003)(Eraky et al. 2016).

Most studies compared the prevalence of *T. gonddi* infection between epileptic subjects and healthy controls. Babaie et al. compared the seroprevalence of toxoplasmosis in epileptic patients and two control groups, a group of neurological patients without epilepsy and another group of healthy controls (Babaie, et al. 2017).

We hypothesized that cases of toxoplasmosis-related epilepsy would be concentrated in patients with cryptogenic epilepsy. Thus, when comparing the prevalence in patients with cryptogenic epilepsy with that of other groups, would be easier to demonstrate any possible relation of *T. gondii* infection with epilepsy.

7.1.1. Were epileptic patients correctly classified?

The classification of patients in suspected cryptogenic and secondary epilepsy was based on the usual criteria (Shorvon 2011). First, some genetic causes of epilepsy were excluded by ruling out patients with a family history of epilepsy and any known congenital syndrome. Patients with alterations in brain imaging, history of CNS lesions or diseases, histories suggesting perinatal hypoxic-ischemic encephalopathy or cerebral palsy were classified as secondary epilepsy. But there were some patients diagnosed of rolandic epilepsy and juvenile myoclonic epilepsy, diseases of known genetic influence that were maintained in cryptogenic group.

Patients were classified as suspected cryptogenic epilepsy when there were no alterations in brain imaging tests and no history of CNS lesions, CNS diseases or perinatal complications. In order to rule out febrile seizures, patients with onset of seizures before 6 years of age were excluded of this group.

In this study there are several elements used for the classification of patients that are not completely reliable. The National Center for the Treatment of Epilepsy is not a general hospital, but a monographic clinical center. Therefore, patients' clinical records are limited to the information that the parents have provided, verbally or with the reports provided. There are no medical records that refer to childbirth and perinatal period. It has no radiological facilities. Brain imaging tests were performed at other public and private health centers, often located in adjacent countries, mainly Egypt and Tunisia, leaving only a copy of the report in the medical record. The cultural level of most of the families served is medium and low, so descriptions of medical history are not always very accurate.

In the National Center for Treatment of Epilepsy there was a cognitive evaluation laboratory, where a technician performed cognitive tests on patients in recent years. Thanks to this, an assessment of the intellectual development of the majority of patients is available. This assessment was generally consistent with the degree of clinically evident intellectual disability. The name of the test used in each patient was not initially recorded and it was not possible to verify it later. It has been found that at least the "WISC test" and the "Raven matrix test" were used. For these reasons, the information derived from the degree of intellectual disability of the patients has not been included in this thesis.

Within patients with suspected cryptogenic epilepsy, 21% of patients had a "moderate intellectual disability". The term "moderate intellectual disability" corresponds to an intellectual quotient (IQ) between 35% and 55% of normal (100%), a really important deficit (American Psychiatric Association 1994). The degree of cognitive deficit found in children with idiopathic epilepsy and cryptogenic epilepsy in developed countries is generally very mild. The IQ found in the majority of the studies performed in these patients are within the normal range (IQ ≥85) or are kept within the limits of the Intellectual Function Limit (IQ of 71-84) (Reijs et al. 2007; Van Mil et al. 2008; Oostrom et al. 2005). In Connecticut (US), only 2% of patients with idiopathic epilepsy and cryptogenic epilepsy had IQs below 60. In contrast, in children with secondary epilepsy, major cognitive deficits were more frequent (36%) and more severe (Berg et al. 2008). Therefore, we suspect that some patients classified as cryptogenic epilepsy

may not be, making it more difficult to detect any difference between both groups of epileptic patients and with non-epileptic controls.

Lagunju and colleagues studied intellectual development in 40 Nigerian children with newly diagnosed epilepsy, with no known cause of epilepsy (Lagunju et al. 2016). The causes of secondary epilepsy were ruled out exclusively by the antecedents referred by their parents. Assessment of IQ at the onset of epilepsy using the WISC-IV found moderate disability (IQ 35-55) in 27% and severe disability in 3%, together with poor school performance in 47% of cases, prior to the onset of epilepsy. It is likely that in developing countries, without advanced perinatal care and without adequate resources for the early detection of intellectual disability in children, many patients with secondary epilepsy of perinatal origin may be classified as idiopathic or cryptogenic epilepsy.

7.2. Subjects in study

7.2.1. Socio-demographic characteristics

Studies about prevalence of toxoplasmosis targeting epileptic children are scarce. Only Potasman (Israel), El Tantawy (Egypt) and Eraky (Turkey) conducted studies on the prevalence of *Toxoplasma gondii* infection in children with epilepsy (Potasman et al. 1995)(Labeeb El-Tantawy et al. 2013)(Eraky et al. 2016). Ngugui analysed separately the prevalence of toxoplasmosis among children under 18 years of age in their study in 5 Sub-Saharan African countries, the largest study in children to date, with 416 cases and 599 controls, but did not found any significant differences in the prevalence of toxoplasmosis between both groups (Ngugi et al. 2013).

Previous studies included children younger than 6 years, in contrast to our study. Children with onset of seizures before 6 years of age were excluded in the cryptogenic epilepsy group, in order to eliminate cases with secondary epilepsy and simple febrile seizures.

The case and controls were similar in terms of socio-demographic characteristics; there were insignificant differences between the three groups studied in terms of age, sex and

residence. In the present study, epileptic patient's residence was higher in rural areas (44%) than in urban ones (26%).

The male / female ratio of children included in this study was 1.5: 1. A total of 59% of cryptogenic and 63% of symptomatic epileptics were male. Female were 41% of cryptogenic and 38% of symptomatic epileptic children. Also a higher male rate was present in the study of El Tantawy in Egyptian children (El-Tantawy et al. 2013), but Eraky et al. reported a slightly higher rate of females (Eraky et al. 2016). It is improbable that these small differences does affect to the results.

7.2.1. Epileptic patients

Study included a total of 148 epileptic participants followed up for epilepsy at National Centre for treatment of Epilepsy. These patients were further divided into two subgroups on the basis of epileptic cause; suspected cryptogenic epilepsy 92 (31%) considered as cases **(group A1)** and symptomatic epilepsy 56 (18.8%) considered as controls **(group A2)**. Mean age was slightly higher in cryptogenic epilepsy patients than in symptomatic epileptics (11.7 and 10.4 respectively). Small mean age differences should not influence comparisons between groups.

7.2.2. Cryptogenic epilepsy

In children with cryptogenic/idiopathic epilepsy cognitive function is usually within normal range but tends to be lower than the general population. Impaired cognitive function in epileptic children is associated with young age at epilepsy onset, underlying pathology, anti-epileptic drugs (AEDs) use (Berg et al. 2008), frequency of seizures and type and duration of epilepsy (Lopes et al. 2013).

In our study, almost 22% had moderate cognitive impairment. This finding was surprising. Her motor and neuroimaging tests were normal. Probably, the neuroimaging studies performed had very limited value in the establishment of a specific diagnosis. We believe that that some of these patients could have symptomatic epilepsies of perinatal origin. Without a thorough record of their perinatal history, some patients with symptomatic epilepsy could be classified as cryptogenic or idiopathic epilepsy.

7.2.3. Symptomatic epilepsy

Fifty-six children with symptomatic epilepsy were considered as controls. These patients had a history suggestive of underlying brain insult or had abnormal brain imaging. Inherited factors and abnormal perinatal history increases the risk of developing epilepsy by 3.2-folds (Senanayake & Román 1993). Therefore, complications in *perinatal period* which start from labor, through delivery, and up to the end of the first week after delivery were also taken in consideration in questionnaire. History of *prenatal period* was excluded from the study. The aetiologies of symptomatic epilepsy were birth related cause in 36% followed by prematurity and meningitis 23%, 18% respectively, close to those of other similar studies in neighbour countries. In one study, the causes of symptomatic epilepsies in Saudi Arabia were pre or perinatal encephalopathy, head injury, childhood neurological infection and stroke (Al Rajeh et al. 2001).

Only 9 patients (16%) had normal brain imaging (Table 8). The majority of children in this group (77%) had moderate to severe cognitive impairment. Cognitive function regarded as mildly impairment in 23%, in concordance with other studies reported in Africa (Abas et al. 2017). In children with symptomatic epilepsy, the level of cognitive function is strongly associated with underlying brain injury, neurologic problems and early onset of seizures (Andersen et al. 2008).

7.2.4. Non epileptic subjects

We selected 150 non epileptic subjects who were otherwise neurologically normal. Included 81 (54%) male and 69 (46%) girls aged from the same geographic distribution and of approximately the same age. The mean age was (11 ± 3.3) years, Nearly the same proportion of participants were from rural, sub urban and urban areas 46 (31%) and 52 (35 %) respectively. Exclusion criteria for controls were a history of epilepsy in their first degree relatives It should be noted. The controls were enrolled from children attending the Tripoli Medical Centre for causes other than epilepsy. These would include patients receiving a venipuncture for medical reasons and an aliquot of this sample would be requested for use as the control serum.

7.3. Toxoplasmosis serology

In current study, the overall seroprevalence of *T. gondii* infection among Libyan children was close to expected, with 29% of IgG positivity. The seroprevalence recorded in this study was lower than the values previously reported for schoolchildren of nearly similar ages (7-18 years) in Libya. Khadre and El Nageh (1987) reported a seropositive rate of 43.7% (Khadre & El Nageh 1987). Toxoplasma seroprevelance in mentally retard Egyptian children was 43.75% (Amrei et al. 1999), which is higher than in the present study. Furthermore the overall prevalence raised from 24.5% at ten years to 52.1% at 20 years of age in Tunisia (Bouratbine et al. 2001). Seroprevalance of toxoplasmosis among Iranian children was 10% (Ali et al. 2007) and Greek 6-15 years old children had an infection rate of around 11% (Frydas et al. 2000), both are lower than our findings.

The study showed that 12% of children had high serum anti-TG IgG antibody levels (titers ≥50 IU/ml). High titers of anti-*Toxoplasma* antibodies are regarded as suggestive of the occurrence of either recent infection and (Kook et al. 1999), or reactivations of parasite from encysted bradyzoites to circulating tachyzoites (Fu et al. 2014).

7.4. Factors related to *Toxoplasma gondii* infection

Toxoplasma infection is related to several factors including socioeconomic level, nutritional habits, age and rural or urban setting (Spalding et al. 2005).

Generally, children are particularly more vulnerable to toxoplasmosis infection due to their behavioural habits. They have higher environmental exposure and a lack of awareness for avoiding these risk factors such as playing in water, soil, eating various raw foods, or contact with pets. Hence they are an ideal target group to investigate toxoplasmosis prevalence (Fan et al. 2012).

7.4.1. Socio-demographic factors

Sero-epidemiological surveys have revealed varying degree of prevalence in terms of different geographical settings and the risk factors for acquiring the disease age distribution, climate and socio-economic status (Bahia-Oliveira et al. 2003).

We have not found significant relation between socio-demographic factors and prevalence of *Toxoplasma gondii* infection in studied children. The same result was obtained when we include the doubtful results as positives in the analysis.

7.4.1.1. Age

Seroprevalence of toxoplasmosis is known to increase with age that is a reflection of increasing risk of exposure with age (Jones et al. 2009). In this study, there were not significant changes in prevalence in studied patients. Seroprevalence was 32% in 12-<18 year-old group and 28% in children aged 6-<12 years. Chung-Jung Fu et al. (2014) observed that infection rates declined with age (Fu et al. 2014).

In our study, mean anti-Toplasma IgG titter was slightly higher in age group of 12-<18 years old (21.2 IU/ml) than in age group 6-<12 years old (16.6 IU/ml), but there was no significant differences. Higher age group was positively related to anti-Toplasma IgG titers >75 UI/mL in logistic regression analysis.

In consistant to our study, Babaie et al. (2017) reported that the IgG titer of anti-*T. gondii* antibodies significantly increased with age, with Pearson's correlation coefficient value of +0.254 (Babaie et al. 2017). The IgG titer for ≤ 30, 31-40, 41-50 and ≥ 51 years of age groups were found as 44.6 ±76, 54.4 ±74, 74.8 ±77 and 106.3 ±84 UI/mL respectively. In contrast to another study among primary schoolchildren in Taiwan, that found significant increased titer of anti-*Toxoplasma gondii* antibodies in younger age group (Fu et al. 2014).

7.4.1.2. Gender

Our study does not found significant gender difference in seroprevalence (boys 30%, girls 28.9%). It might be because both genders are having similar routes for acquisition of *T. gondii* infection (Fan et al. 2012). Although, Akyol et al. found increased prevalence in Iranian boys (Akyol et al. 2007).

7.4.1.3. Area of residency

Sero-positivity was almost the same among residents in rural, suburban and urban areas; 31%, 30% and 27% respectively. This coincides with Zibaei et al. (2011), who did not

find significant differences between the seroprevalence of T. gondii infection in rural areas compared to urban areas (Zibaei et al. 2011). This can be explained by various risk factors associated with *T. gondii* infection that have the same distribution in urban and rural areas. This is in consistent with other studies in north Africa (el-Nawawy et al. 1996)(El-Tantawy et al. 2013).

7.4.2. Life style risk factors of *Toxoplasma gondii* infection

In the bivariate analysis, five variables were identified as possible risk factors for toxoplamosis (Table 13 and 14): Eating raw meat, Eating raw vegetables, Source of drinking water, Contact with soil and Contact with cats (house cat, neighbourhood cat). Further analysis using multivariate logistic regression revealed that all variables found statistically significant in the bivariate analysis kept the statistical significance in the multivariate model (Table 16).

7.4.2.1. Raw meat or under cooked meat consumption

Raw or undercooking meat consumption was positively associated with *Toxoplasma gondii* infection in our study. Children who eat raw meet were 3.36 times at higher risk of being seropositive than children who were not. In Libya, lamb, goat, beef, camel and chicken meat are commonly used. Pork is never used due to religious ban. It is well documented that lamb and goat meat are sources of *T. gondii* (Dubey 2000). Generally, thorough cooking is always preferred in Libya. However, raw meat is also consumed in traditional food as "kaedid" which is dry salty raw meats and undercooked meat at a barbecue. Consumption of raw meat or undercooked meat can serve as a route of infection in our study. This result is similar to that done in children from a Brazilian rural area (Souza et al. 1987)**.** While ingestion of raw meat appeared to play no role in transmission in Panamanian children (Frenkel et al. 1995)**.**

7.4.2.2. Raw vegetables (how often the child eats vegetables)

Contaminated fruit and vegetables probably by cat faeces and poor hand hygiene also are important in parasite transmission in our study. Children who frequently eat raw vegetable were 4 times more likely to be seropositive compared to who didn't eat it.

Which coincides with studies from Cameroon and China (Njunda et al. 2011)(Xin et al. 2015).

In epileptic children, frequent eating of raw vegetables had an increased excess risk of *Toxoplasma gondii* infection, with odds ratio of 20.66 (Table 26: OR 20.66; 95% CI 3.7-115), thus being a major risk factor of toxoplasmosis infection in our study.

7.4.2.3. Source of drinking milk

Non-processed milk from several animals is a potential source of *T. gondii* transmission (Sacks et al. 1982)(Dubey et al. 2014). Contamination of milk might be due to dirty production techniques. Or it may be related to other lifestyle risk factors such as eating undercooked meat or hygienic habits (Cook et al. 2000). Generally, most of population consumes pasteurized milk. However, the consumption of raw milk in rural and some suburban areas is common. In this study, most of the children were from rural and suburban areas and consumption non-pasteurized milk was recognized in 17% of participants. Our data (Table 13) showed that raw milk probably played small role in parasite transmission in our patients. It was not significantly related to seroprevalence of toxoplasma gondii infection in bivariate analysis nor was it an independent risk factor in logistic regression analysis.

7.4.2.4. Personal hygiene (hand washing habit)

Poor hygiene habits at food handling and prior to eating, there also appears to increase the probability of human infection (Baril et al. 1999). We didn't found that personal hygiene habits were a risk factor associated with *T. gondii* seroprevalence in our sample. But its effect logically would be related to other risk factors such as contact with soil or pets.

7.4.2.5. Contact with soil

In agreement with our result contact with soil has been strongly associated with *T. gondii* infections, probably because it is a direct source of oocysts (Spalding et al. 2005). Contact with soil is difficult to avoid in childhood period, where they carry out their play activities. The children were asked to how frequently they washed their hands

after having contact soil. Accordingly, children who had contact with soil and not always having hand washing practices were 3.46 times at higher risk of infection than those who did not have contacts or clean their hands after contact with soil. This remained an important independent risk factor within epileptic children, with OR of 11.3 (95% CI 3.54-36.18) as showed in (Table 26).

7.4.2.6. Contact with cats

In Libya, to have cats or dogs as pet is forbidden but there is a relatively high population of stray cats. Indeed, 17 % of the total study population reported having cats at home. They allow their cats to deposit faeces outside home. Also these cats are fed with raw meat and lack routine veterinary care. Most cat owners neglect of preventive measures (i.e., washing hands). Contact with cats has not been found to be a risk factor as important as contact with cat faeces or soil contaminated with cat's faeces (Cook et al. 2000). In this study, we found that that contact with cats increased the risk of *T. gondii* infection by 2.81-fold. It seems to be a main risk factor (Montoya & Liesenfeld 2004). Cat ownership and contact with neighbourhood or stray cats near the household were strongly associated with infection (Table 14 and 16). This corroborates study performed in Brazilian urban children (Dattoli et al. 2011). Presence of neighbourhood cats explained most of the effect of "contact with cats" in multivariate analysis; but if this variable is omitted in the model other related variables of "contacts with cats" are selected.

7.4.2.7. Contact with dogs

The role of the dogs and other farmer animals is not entirely clear. However, they may act as mechanical vectors (Frenkel and Parker, 1996). Most of the dogs are either stray dogs or of farm dogs, as living in a household with a dog is not a common tradition in Libya. This explains why child contact with dogs was insignificant in our study and it was not related to *T. gondii* infection.

7.4.2.8. Source of drinking water

In Libya it is common for rural households to have wells to extract water for their own consumption. Rainwater deposits are also frequent, both in cities and in rural areas.

Water from these alternative sources is generally not filtered or chlorinated. However, paradoxically, this study has shown that the availability of potable water from wells or rainwater reservoirs has been linked to a lower prevalence of *Toxoplasma gondii* infection. This protective effect of drinking water from sources other than the public water supply network has been confirmed in both bivariate and multivariate analyses (OR 0.37; 95% CI 0.19-0.72). It may be due to its association with some other uncontrolled factors, but it must be confirmed in the future.

In Libya, the public water supply network is mainly based on a complex network of large-scale channels and pipelines that carry water extracted from the southern aquifers, by means of wells, to the northern coastal cities. This important infrastructure has suffered great damage during the difficult situations that have occurred in the country in the last recent years. To such an extent, that the provision of potable water in areas of Tripoli and other northern cities is often compromised.

7.4.3. Other factors

7.4.3.1. Blood transfusion

Although very uncommon, blood transfusions can cause infection via tachyzoites containing blood (Tenter et al. 2000). In Egypt found that more than 50% of blood donors have been seropositive for Toxoplasma (Elsheikha et al. 2009).

In our study, only two of twelve children who had received a blood transfusion, were toxoplasma seropositive (17%). This finding is supported by a previous study (Nissapatorn et al. 2003).

7.5. Relations of toxoplasmosis and epilepsy

Epilepsy is a major chronic neurological disorder burden in developing countries, particularly Africa and South America, where a number of infections are also predominantly reported as epilepsy aetiology. Parasitic infections are important causes of mental and other neurological disorders in these countries (Ngoungou et al. 2015). Toxoplasmosis and toxocariasis have been suggested as a significant hazard for epilepsy development and needing confirmation. There is limited data available on this topic.

The possible impact of toxoplasmosis and its role in the occurrence of epilepsy is not well defined up to date. Tissue cysts of toxoplasma are located in numerous anatomical sites including the brain. Bradyzoites inside tissue cysts may reactivate, exit cyst, transform in tachyzoites and to infect new cells. *Toxoplasma gondii* inside neurons can directly induce changes in neurotransmisors and transmembrane potential which lead to triggering seizures (Brooks et al. 2015); or this parasite can to acts indirectly, through activation of the immune system and inducing local inflammatory response. Localized inflammation may produce microglial scars (granuloma) which may lead to focal seizure (Palmer 2007). The alternative explanation to an increased prevalence of T. gondii infection in epileptic patients or patients with cryptogenic epilepsy may be that these subjects could be more vulnerable or more prone to *T. gondii* infection (Stommel et al. 2001).

As reviewed in the introduction, a few studies from Israel, USA, Turkey, Iran, Egypt and various countries of sub-Saharan Africa investigated the causal relationship between toxoplasma infection and the cryptogenic epilepsy. A total of 13 studies were conducted, five studies out of thirteen were reported as a failure to find significant relationship between toxoplasmosis and epilepsy as summarized in Table 3. Most of these studies had small sample sizes. However, two published meta-analysis exploring linkages between toxoplasmosis and epilepsy concluded that this relation does exits (Palmer 2007) (Ngoungou et al. 2015).

Even if there is an indication of link reported between epilepsy and Toxoplasmosis, there is uncertainty about whether this involves causality. Uzorka and Arend (2017) performed a systematic review of medical literature on this topic and the Bradford Hill criteria for causality were used to score selected articles (Höfler 2005). Authors found evidence of causality in 3 medical articles, two case reports and one case-control study. More definite proof requires further research, e.g. by performing Toxoplasma serology in all de novo epilepsy cases (Uzorka & Arend 2017).

Anti-Toxoplasma IgG antibodies were found in 35.2% in children with cryptogenic epilepsy in our study but similar rates of Toxoplasma seropositivity were found in symptomatic epileptic patients (29.4%) and in control subjects (29%).

This percentage is similar to seroprevalence in epileptic children in the study conducted by Ngugi et al. (2013) in Sub-Saharan Africa (35.4%) (Ngugi et al. 2013). In Egypt

there are contradictory data about prevalence of toxoplasmosis in epileptic children, from 60.6% (El-Tantawy et al. 2013) to 20% (Eraky et al. 2016).

Our results did not found direct association between toxoplasmosis prevalence and cryptogenic epilepsy, as there was no statistical significant difference between patients with cryptogenic epilepsy, symptomatic epilepsy and non-epileptic controls. But there were some indirect observations that can support this relation.

Anti-Toxoplasma IgG titters (Figures 40 and 44) were statistically significantly higher in cryptogenic epilepsy patients (27±4.3 IU/ml) than in symptomatic epilepsy patients (15.3±5.8 IU/ml) and non-epileptic subjects (16.1±4 IU/ml). Similar results have been observed when comparing the percentage of patients with high anti-Toxoplasma IgG titers. Percentage of subjects with anti-Toxoplasma IgG >75 IU/ml was higher in cryptogenic epilepsy patients (19.4%) than in those with symptomatic epilepsy (6.6%) and non-epileptic subjects (6.7%) (Figure 41 and 45). Elevated antibody titers could reflect recent infections or could serve as a proxy for estimating the level of exposure, as antibody levels would be elevated due to repeated infections or reactivations. (Kamuyu et al. 2014).

Higher titers of anti-toxoplasma IgG were also found in epileptic patients by Stommel et al. in US (Stommel et al. 2001) when comparing epileptic adults and healthy controls, althoug percentage of seropositivity was not statistically different between both groups.

Other authors who have studied different neurological abnormalities attributable to toxoplasma gondii infection have also found higher IgG titers in studied cases than in the controls, although the seroprevalence between cases and controls was not different. These results have been obtained when studying the possible relationship of toxoplasmosis with psychiatric disorders, schizophrenia and depression (Duffy et al. 2015) , and work and traffic accidents (Alvarado-Esquivel et al. 2012)(Galván-Ramírez et al. 2013)(Flegr et al. 2009). There has been demonstrated a direct relation between antibody titers against *T. gondii* and risk of traffic accidents (Flegr et al. 2002).

7.5.1. Type of seizures

On the basis of the ILAE classification, considering both EEG and clinical data, 93 patients (63 %) had generalized seizures while 55 patients (37.2%) presented focal

seizures. If one patient presented both, partial and generalized seizures it was considered as focal seizures. In comparison, focal seizures were less common in patients with suspected cryptogenic epilepsy (33.7%), while partial seizures with and without secondary generalization was more frequent in symptomatic epilepsy (42.4%).

Our study showed that seroprevelance of toxoplasmosis was significantly higher in children with focal seizures (47.1%) than those with generalized seizures (29%) (Table 20). After adjusting for the effects of other variables, using a multivariable logistic regression model, when the analysis was restricted to patients with cryptogenic and symptomatic epilepsy (148 subjects) we found that the association between seropositivity to *Toxoplasma gondii* and focal epilepsy was more strong and significant, with an adjusted OR of 2.67 (95% CI: 1.28-5.54; P = 0.009).

But this increased prevalence of toxoplasma infection in subjects with focal epilepsy was seen both in patients with cryptogenic and secondary epilepsy (Figures 51 and 52) and in patients with focal epilepsy with abnormal brain MRI (Figure 54). The finding of increased seroprevalence of toxoplasmosis in patients with focal seizures diagnosed with symptomatic epilepsy or with altered cerebral MRI may suggest two things, either this relationship is false or accidental or toxoplasma infection may play an etiological or pathogenic role also in the development of epilepsy in some patients with previous brain lesions. The finding that toxoplasma infection decreases the epileptogenic threshold in laboratory animals could justify this relationship (Babaie et al. 2017).

On the other hand, most of the lesions diagnosed in the brain MRI were periventricular leukomalacia and this was also the most frequent finding in patients diagnosed with symptomatic epilepsy and presenting focal seizures (Table 22). Periventricular leukomalacia was the only finding in MRI in 8 of the 18 cases of patients with symptomatic epilepsy, focal seizures and seropositivity for T. gondii. But not all children with periventricular leukomalacia develop seizures; these appear only in 26% to 33% of cases; and mainly among those with neonatal seizures, major neurological lesions and delayed neurological development (Humphreys et al. 2007)(Ekici et al. 2013). There is a theoretical possibility that some of these patients develop epilepsy for other reasons, but most likely this epilepsy will be blamed on their perinatal history if no other cause is demonstrated.

There are isolated case reports of focal seizures related to *Toxoplasma gondii* infection (Michałowicz et al. 1989) or as initial manifestation of AIDS related brain toxoplasmosis (Özkaya et al. 2006). But it has not been explored in all preceding studies, or authors did not report the types of seizures presented (Yazar et al. 2003)(Babaie et al. 2017)(El-Tantawy et al. 2013)(Ngugi et al. 2013).

Most studies in industrialized countries have reported a higher proportion of primary generalized seizures. In contrast, in developing countries the trend seems to be a higher frequency of partial seizures, usually secondarily generalized (Senanayake & Roman 1993). *Taenia solium* and *T. canis*, another parasitic zoonosis, were considered the main cause of neurological diseases in developing countries of Latin America, Africa and Asia. It has been suggested that both *T. solium* and *T. canis* may explain the higher incidence of focal epilepsy in developing countries. This significant positive association between partial epilepsy and seropositivity of both parasites has been confirmed by two case-control studies carried out in rural Bolivia (Nicoletti et al. 2002) and in Burundi (Quet et al. 2008).

7.5.2. Control of seizures

The main aim of epilepsy therapy is to make the patient totally seizure-free or to reduce the duration, frequency and severity. However, More than 10% of patients develop medically poor controlled epilepsy (Bourgeois 1995).

In our study, cryptogenic epilepsy had significantly better control than patients with symptomatic epilepsy. Complete seizure control (seizure-free for at least 2 years) to antiepileptic drug (AED) was estimated in 15% of patients with cryptogenic epilepsy, nearly 23% were with good control (with absence seizure in 6 months) and 40% responded partially (some seizures in 6 months). On the other hand in the symptomatic epileptic group, there was less than 5 % of complete response; 20 % responded partially and well control could be observed in only 5%. There were some differences in number of antiepileptic drugs (AED) that received children with symptomatic epilepsy and cryptogenic epilepsy. Monotherapy was used in similar frequency, 55.5% in patients with symptomatic epilepsy and 58.7% in patient with cryptogenic epilepsy. But 25% of patients with symptomatic epilepsy needed three or more drugs, versus 9.8% in the cryptogenic epilepsy group.

There was no relationship between epilepsy control and *T. gondii* infection (Table 18). A significant relationship was not found when an independent analysis of patients with symptomatic epilepsy and cryptogenic epilepsy was performed.

There are isolated published cases of patients with congenital toxoplasmosis and difficult-to-control epilepsy (Jeong et al. 2015)(Rheims et al. 2011). We have not found in the medical literature studies on the possible influence of *Toxoplasma gondii* infection in the control of epilepsy with which to compare these results.

7.6. Final remarks

Toxoplasmosis is the most prevalent human parasitic infection in the world. Acquired toxoplasmosis is considered a banal infection in non-immunocompromised individuals, but parasitic tropism by the central nervous system, especially by neurons, has aroused the interest of researchers in recent years.

The possible pathogenic role of toxoplasmosis in the development of epilepsy, psychiatric disorders and behavioral changes is still open to debate. In recent years important advances have been made on the physiological basis of the epileptogenic potential of *Toxoplasma gondii* in laboratory animals, but their transfer to the clinic in humans has not yet occurred.

The importance of toxoplasmosis as a possible source of epilepsy will depend on the prevalence of this infection in each population. It will be low in developed countries and in those with low toxoplasmosis prevalence and may be more important in developing countries with a high prevalence of this infection.

STUDY LIMITATIONS

8. STUDY LIMITATIONS

The present study covered the western area of Libya, as this is the catchment area of the National Center for Treatment of Epilepsy and the Tripoli Medical Center. It includes the north, the northwest and several regions of south-western Libya. The results shown here do not therefore represent the eastern areas of the country.

Not all eligible patients could be included in the study. Interviews were conducted after medical consultations and some patients were unwilling to remain in the hospital to participate in the study. In addition, some children could not be included in the study because the adults who accompanied them were unaware of some of the birth details and perinatal history required for the interview. These are potential sources of selection bias.

On some occasions, the reporting quality of the accompanying adults was low as patients and relatives were tired after the consultations. However, it is estimated that this was uncommon and did not have an impact in the results of this study

Our inclusion criteria were based on a commonly used, and standard, definition of cryptogenic epilepsy and it is possible that some of them were missed. We have reasons to think that among patients considered cryptogenic epilepsy were some who actually had symptomatic epilepsy. This adversely affects the study's ability to discriminate between the prevalence of Toxoplasma infection between both groups and non-epileptic controls.

Some patients had doubtful titers of IgG anti Toxoplasma gondii, according to the reference values of the laboratory that manufactured the Kitts. The correct way to resolve this situation is to repeat the analyses with a reference test, such as the Sabin-Feldman test or immunoblotting. However, it was not possible to repeat the serology of doubtful cases.

In most statistical calculations patients with doubtful titers of antibodies have been excluded. Therefore, the total number of cases has been reduced, slightly decreasing the contrast power to the tests carried out.

CONCLUSSIONS

CONCLUSIONES

9. CONCLUSIONS/CONCLUSIONES

9.1. CONCLUSIONS

9.1.1. Introduction

There are few studies evaluating the association between T. gondii infection and epilepsy. These studies show contradictory results, with some finding a positive association, while others find no relationship. There are no available data from Europe, Latin America, or most Asian countries except Israel, Turkey, and Iran.

The results presented here, despite the mentioned limitations, indicate that toxoplasmosis can be considered a plausible risk factor for epilepsy:

9.1.2. Conclusions

- We have not found significant differences in prevalence of *T. gondii* infection between epileptic children and non-epileptic controls.
- We have not found significant differences in the prevalence of *T. gondii* infection among patients with suspected cryptogenic epilepsy and patients with symptomatic epilepsy and controls without epilepsy.
- Patients with suspected cryptogenic epilepsy presented higher titers of IgG anti T. gondii than control groups and a higher percentage of cases with high titers of IgG. This may lead to indirect evidence in favour of T. gondii infection being able to play a pathogenic role in the development of epilepsy in these patients.
- Patients with focal epilepsy showed greater prevalence of T. gondii infection than patients with other types of seizures and non-epileptic controls. This supports that focal epilepsy may be related to T. gondii infection.
- There was no relation of toxoplasma gondii infection to the degree of control of epilepsy.
- The seroprevalence of *T. gondii* infection among all studied children, residing in western Libya and aged 6 to 18 years, was found to be 29.4% and 5.4% had doubtful IgG titers.

- Excluding subjects with doubtful IgG titers, the seroprevelance of *T. gondii* infection in studied children with cryptogenic epilepsy, symptomatic epilepsy and non-epileptic controls were 35.2%, 29.4% and 29.4% respectively.
- The risk factors that most strongly were associated with acquired *T. gondii* infection in children were eating uncooked dried meat or undercooked meat, eating raw vegetables, contact with soil, contact with cats and the presence of neighbourhood cats.
- Taking drinking water from wells and rain deposits was associated to a paradoxical lower prevalence of *T. gondii* infection.

9.2. CONCLUSIONES

9.2.1. Introducción

Existen pocos estudios que evalúen la asociación entre la infección por T. gondii y la epilepsia. Estos estudios muestran resultados contradictorios, con algunos encontrando una asociación positiva, mientras que otros no encuentran ninguna relación. No hay datos disponibles de Europa, América Latina ni de la mayoría de los países asiáticos, con la excepción de Israel, Turquía e Irán.

Basándose en los datos disponibles, con sus obvias limitaciones, sigue siendo apropiado considerar la toxoplasmosis como un posible factor de riesgo para el desarrollo de epilepsia.

9.2.2. Conclusiones

- No hemos encontrado diferencias significativas en la prevalencia de infección por T. gondii entre niños epilépticos y controles no epilépticos.
- No hemos encontrado diferencias significativas en la prevalencia de infección por Toxoplasma gondii entre los pacientes con sospecha de epilepsia criptogénica y los pacientes con epilepsia sintomática o los controles sin epilepsia.
- Los pacientes con sospecha de epilepsia criptogénica presentaron mayores títulos de IgG anti Toxoplasma gondii que los grupos control y un mayor porcentaje de casos con altos títulos de IgG. Esto puede considerarse una evidencia indirecta a favor de que la infección por Toxoplasma gondii pueda desempeñar un papel patógeno en el desarrollo de epilepsia en estos pacientes.
- Los pacientes con epilepsia focal mostraron mayor prevalencia de infección por Toxoplasma gondii que los pacientes con otros tipos de convulsiones y los controles no epilépticos. Esto sugiere que la epilepsia focal puede estar relacionada con la infección por Toxoplasma gondii.
- No hubo relación entre la infección por *Toxoplasma gondii* y el grado de control de la epilepsia.
- La seroprevalencia de la infección por *T. gondii* en el grupo completo de niños estudiados, residentes en la zona occidental de Libia y con edades comprendidas

entre los 6 y 18 años, era del 29,4% y un 5,4% adicional tenían títulos dudosos de IgG.

- Excluyendo a los sujetos con títulos dudosos de IgG, la seroprevalencia de la infección por T. gondii en los niños estudiados con epilepsia criptogénica, epilepsia sintomática y controles no epilépticos fue del 35,2%, 29,4% y 29,4%, respectivamente.
- Los factores de riesgo que más fuertemente se asociaron con la infección adquirida por *T. gondii* fueron comer carne seca cruda o carne poco cocinada, comer verduras crudas, el contacto con el suelo, el contacto con gatos y la presencia de gatos en el vecindario.
- Tomar el agua potable de pozos y depósitos de lluvia se asoció paradójicamente a menor seroprevalencia.

9.3. Recommendations

These findings should be checked with studies of larger sample size and with a better classification of epileptic patients. More specifically, a careful investigation of the personal history, a prospective assessment of the cognitive status of the patients and the diagnostic techniques necessary for a correct classification should be carried out.

Sufficient resources should be available to confirm the serological status of the cases with questionable results, repeating studies with reference techniques such as immunoblot or Sabin-Feldman.

These studies should be done in a larger sample to improve our knowledge about the risk factors of human exposure to *T. gondii*, as there were no adequate studies on the modes of transmission in Libya.

The results of the present study indicate the need to implement prevention methods and control measures against Toxoplasma infection in Libya. In general, there is a lack of awareness about toxoplasmosis, its risk factors and symptom. Adequate health education programs are needed, especially among children. Teachers and parents play an important role in guiding children to maintain good personal hygiene and healthy eating habits.

Strategies should be designed for the control of stray cats. Improvements in sanitation and an epidemiological surveillance policy are needed to avoid an increase in toxoplasmosis cases.

Epileptic children and their families suffer from stigma and discrimination, especially women. More attention should be paid to these groups, including psychological, social, legal and family support.

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10. REFERENCES

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APPENDIX

11. APPENDIX

I. Informed consent forms

A. Consent form for CASES

استمارة معلومات للحالات(Case)

اسمى دكتورة فاطمة الشيبانى. أنا طبيبة أقوم بالإعداد لدراسة لدرجة الدكتوراه بقسم طب الاطفال كلية الطب بجامعة أوتونوما بمدريد(اسبانيا). أنا أقوم بإجراء بحثي عن عدوى تسمى داء المقوسات وما هي أو كيفية علاقتها بمرض الصرع. . تجدون مرفق نشرة توضح كيفية الحصول على العدوى.

من اجل معرفة عدد الأطفال الذين ستكون نتيجة فحصهم إيجابية. إننا نود أن نأخذ عينة صغيرة من دم طفلك لعمل ذلك و الإجابة على أسئلة قليلة عن تاريخ مرض طفلك . إذا لديك الرغبة في معرفة نتيجة فحص ، يمكنك الاتصال بالدكتورة بهيجة أبو حجر وهي المسئولة التى ستقوم بالتوضيح لك.

لن تكلفك هذه الدراسة أي شئ . المعلومات التي ستدلى بها ستظل سرية للغاية إلا للباحثين فقط. ولك مطلق الحرية. فالمشاركة لن يؤثر على علاقتك بالعاملين والأطباء بالمركز الصحي أو الرعاية التي يتلقاها طفلك. إذا لديك الرغبة في أن ينضم طفلك إلى هذه الدراسة، الرجاء التوقيع في المكان المخصص

هل هنالك أي أسئلة يمكن أن نجيب عليها لتساعدك على فهم افضل للدراسة
أؤكد أنني قد تم منحى المعلومات عن الدراسة و أيضاً لان اسأل أسئلة

ارغب في أن يشارك طفلي_____في الدراسة
اسم الوالد/ ألوصى_____

التوقيع_____ التاريخ_____

English translation:

Dear Parents

Let me introduce myself, I am Dr Fatima Alshaibani, a PhD student at the University Autonoma of Madrid, Spain. I am conducting a study about an infection called Toxoplasmosis and how this is linked to epilepsy of unknown origin.

Your child has been selected to take part in a survey about it for research purposes. However knowing if it is common in children with seizure will help us increase awareness of the dangers of this illness. Attached is a sheet which describes how this infection is acquired and steps of protection.

We are studying a specific blood test for diagnosis of Toxoplasmasis in order to measure how many children have a positive test. We would like to take a small sample of your child's blood to do this and would request you to answer a few questions on the history of your child's illness.

If you wish to know the test result of your child, you are requested to contact to your doctor and he will explains this result for you.

Whether you participate or not in this study this will not affect your relationship with

health centre staff and your doctors or the care your child receives.

Are there any question we can answer to help you better understand the study?

If you would like your child to join this study please sign in the space below.

I confirm I received information about the study.

I wish my children _____ to take part in this study.

Name of parents/ guardians:

Signature:

Date:

B. Consent form for CONTROLS:

استمارة معلومات للحالات المحصورة(Case Control)

اسمى دكتورة فاطمة الشيبانى. أنا طبيبة أقوم بالإعداد لدراسة لدرجة الدكتوراه بقسم طب الاطفال كلية الطب بجامعة أوتونوما بمدريد(اسبانيا). أنا أقوم بإجراء بحثي عن عدوى تسمى داء المقوسات وما هي أو كيفية علاقتها بمرض الصرع.

إننا نقوم في الأساس بدراسة الأطفال المصابين بالصرع ، ولكننا نحتاج لعينة من الأطفال الغير مصابين بداء الصرع لمعرفة إذا ما كانت هنالك أي فرصة لحصولهم على هذه العدوى. إننا نود اخذ عينة من دم طفلك و نطلب منك الإجابة على بعض الأسئلة. فإذا كانت نتيجة طفلك إيجابية فليس بالشيء المؤذى وهذا يعنى أن طفلك لديه حماية من العدوى في المستقبل. إذا كانت سلبية فيمكن أن يكون عرضة للعدوى.

لن تكلفك هذه الدراسة أي شئ . المعلومات التي ستدلى بها ستظل سرية للغاية إلا للباحثين فقط. ولك مطلق الحرية. فالمشاركة لن يؤثر على علاقتك بالعاملين والأطباء بالمركز الصحي أو الرعاية التي يتلقاها طفلك. إذا لديك الرغبة في أن ينضم طفلك إلى هذه الدراسة، الرجاء التوقيع في المكان المخصص
إذا لديك الرغبة في معرفة نتيجة فحص ، يمكنك الاتصال بالدكتور بشير العلاقى وهو المسئول الذى سيقوم بالتوضيح لك.

هل هنالك أي أسئلة يمكن أن نجيب عليها لتساعدك على فهم افضل للدراسة
أؤكد أنني قد تم منحى المعلومات عن الدراسة و أيضاً لان اسأل أسئلة

ارغب في أن يشارك طفلي_____في الدراسة
اسم الوالد/ ألوصى_____

التوقيع_____ التاريخ_____

تجد مرفق نشرة توضح ماهو داء المقوسات وكيفية الوقاية منه

English translation:

Dear Parents

Let me introduce myself, I am Dr Fatima Alshaibani, a PhD student at the University Autonoma of Madrid, Spain. I am conducting a study about an infection called Toxoplasmosis and how this is linked to epilepsy of unknown origin.

Your child has been selected to take part in a survey about it for research purposes. However knowing if it is common in children with seizure will help us increase awareness of the dangers of this illness. Attached is a sheet which describes how this infection is acquired and steps of protection.

We are mainly studying children with epilepsy, but need a sample of children without epilepsy to find out if they have chance of getting this infection. These are control children and your child is in that group. We would like to take a small sample of your child's blood to do this and would request you to answer a few questions on the history of your child's illness. We are studying a specific blood test for diagnosis of Toxoplasmasis in order to measure how many children have a positive test. We would like to take a small sample of your child's blood to do this and would request you to answer a few questions on the history of your child's illness.

If you wish to know the test result of your child, you are requested to contact Head of infectious disease department and they will explains this to you.

Whether you participate or not in this study this will not affect your relationship with

health centre staff and your doctors or the care your child receives.

Are there any question we can answer to help you better understand the study?

If you would like your child to join this study please sign in the space below.

I confirm I received information about the study.

I wish my children _____ to take part in this study.

Name of parents/ guardians:

Signature:

Date:

II. Questionnaires

A. Questionaries' for epileptic children

I.A Questioner

A controlled study of anti-Toxoplasma antibody prevalence in Libyan epileptic children

For cases

Number of questioner:

Name of childe:

Date of birth (age):

Sex of childe

Male Female

Residence

Urban Suburban Rural

A) Child past history of:

1. Blood transfusion:
2. Ingestion of raw meat:
3. Source of drinking water: Main supply
   Other source
4. Drinking milk: Raw unpasteurized milk
   Pasteurized milk
5. Eating raw meat:
6. Contact with soil:
7. Good personal hygiene :
8. Contact with cat:
9. House cat
10. Neighborhoods cats
11. Number of cats at home

B) History of seizure

1. Age onset:
2. Frequency in last 6 month:
3. Type of seizure:
4. Type of treatment:
5. Result of EEG: CTSCAN: MRI:

C) PERI/POSTNATAL HISTORY

1. Gestation age:
2. Perital history:
3. Postnatal history:

D) Neuro development history

1. Neurological impairment:
2. Age of starting walk:
3. Age of starting speech:
4. History of speech disorder:
5. Attending school:
6. Age appropriate school:
7. History of learning disability:

E) Antibody test result

B. Questionaries' for controls

I.B Questioner

A controlled study of anti-Toxoplasma antibody prevalence in Libyan epileptic children

For control

Number of questioner:

Name of childe:

Date of birth (age):

Sex of childe

Male Female

Residence

Urban Suburban Rural

A) Child past history of:

1. Blood transfusion:
2. Ingestion of raw meat:
3. Source of drinking water: Main supply
   Other source
4. Drinking milk: Raw unpasteurized milk
   Pasteurized milk
5. Eating raw meat:
6. Contact with soil:
7. Good personal hygiene :
8. Contact with cat:
9. House cat
10. Neighborhoods cats
11. Number of cats at home

C) PERI/POSTNATAL HISTORY

1. Gestation age:
2. Perital history:
3. Postnatal history:

D) Neuro development history

1. Neuro logical impairment:
2. Age of starting walk:
3. Age of starting speech:
4. History of speech disorder:
5. Attending school:
6. Age appropriate school:
7. History of learning disability:

E) Antibody test result

III. Informative sheet

تقدم مع استمارة الموافقة

ما هو داء المقوسات؟

داء المقوسات هو عدوى يسببها طفيلي ميكروسكوبي يسمى التوكسوبلازما قوانداى. معظم الناس الذين يصابون بداء المقوسات لا يمرضون، ولكن بعض الناس يحدث لهم تورم في الغدد الليمفاوية ويشعرون كأنهم مصابون "بالزكام" .

يمكن أن يسبب داء المقوسات مرض حاد للأطفال المصابين بالعدوى قبل الولادة (عندما تصاب أمهاتهم بالعدوى أثناء الحمل) مما يؤدى إلى التشوه الخلقي أو الإجهاض أو في بعض الناس الي ضعف في جهاز المناعة .

كيف يصاب الناس بعدوى داء المقوسات؟

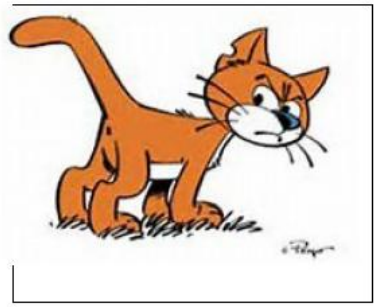
هل يمكن علاج داء المقوسات؟

نعم. هنالك علاج لداء المقوسات. فعندما تصاب المرأة بالعدوى أثناء الحمل يمكن علاجها بالأدوية التي ستحمى طفلها الغير مولود من داء المقوسات. يجب مراقبة الأم والطفل عن قرب أثناء الحمل وبعد ولادة الطفل.

كيف يمكنني تقليل مخاطر الإصابة بداء المقوسات؟

تجنب أكل اللحوم الغير مطهية و اغسل الفواكه والخضروات جيداً قبل أكلها

تجنب شرب الحليب غير المبستر و شرب الماء الذى لم تتم معالجته



تجنب القطط الضالة، خاصة الصغار منها واغسل يديك جيداً بالماء والصابون إذا حدث تعامل مع براز القطط