Seroprevalences of *Toxoplasma gondii* and *Neospora caninum* infections in Jordanian women who had a recent spontaneous abortion

Soroprevalências de infecções por *Toxoplasma gondii* e *Neospora caninum* em mulheres jordanianas que tiveram um aborto espontâneo recente

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Abstract

This cross-sectional study investigates *Toxoplasma gondii* and *Neospora caninum* among 445 recently spontaneously aborted (RSA) Jordanian women using ELISA and indirect fluorescent antibody (at a cut-off value of 1/200) tests, respectively. The type of hospital, age, cat and dog contacts, raw and barbecued meat and wild plant consumption, number of abortions, and stillbirths were tested as independent variables using univariate and multivariate logistic regression analyses. The true seroprevalences were 22.1% for *T. gondii*-IgG, 22.7% for *N. caninum*-IgG, 2.6% for *T. gondii*-IgM, 10.6% for *N. caninum*-IgM, 0% for *T. gondii*-IgG and IgM, 6.7% for *N. caninum*-IgG and IgM, and 4.6% and 0% for both parasite IgG and IgM, respectively. *T. gondii*-IgM-seropositivity was associated with the number of abortions with odds ratios (OR) of 2.4 and eating barbecued meat (OR = 0.12). *N. caninum*-IgG-seropositivity was associated with having a dog in the house (OR = 2.6), and with stillbirth (OR = 0.1). *N. caninum*-IgM was associated with visiting a private-hospital (OR = 2.7). RSA Jordanian women are equally exposed to both parasites with significantly ($p < 0.05$) higher seroprevalence of *N. caninum*-IgM compared to *T. gondii*-IgM suggestive of active infections among RSA women in Jordan.

Keywords: *Toxoplasma gondii*, *Neospora caninum*, seroprevalence, risk factors, abortion, Jordan.

Resumo

Este é um estudo transversal, investigando *Toxoplasma gondii* e *Neospora caninum* entre 445 mulheres jordanianas recentemente abortadas espontaneamente (RSA), usando-se ELISA e testes de anticorpos fluorescentes indiretos (com valor de corte de 1/200), respectivamente. Tipo de hospital, idade, contato com o cão, consumo de carne, número de abortos foram testados como variáveis independentes, usando-se análises de regressão logística univariada e multivariada. As verdadeiras seroprevalências foram 22,1% para *T. gondii*-IgG; 22,7% para *N. caninum*-IgG; 2,6% para *T. gondii*-IgM; 10,6% para *N. caninum*-IgM, 0% para *T. gondii*-IgG e IgM, 6,7% para *N. caninum*-IgG e IgM, e 4,6% e 0% para ambos os parasitas IgG e IgM, respectivamente. A soropositividade para *T. gondii*-IgM foi associada ao número de abortos com "odds ratio" (OR) de 2,4 e ingestão de carne grelhada (OR = 0,12). A soropositividade para *N. caninum*-IgG foi associada à presença de cachorro em casa (OR = 2,6) e natimorto (OR = 0,1). *N. caninum*-IgM foi associada à visita a um hospital privado (OR = 2,7). Mulheres jordanianas com RSA estão igualmente expostas a ambos os parasitas com soroprevalência significativamente (p <0,05) maior de *N. caninum*-IgM, em comparação com *T. gondii*-IgM, sugestivo de infecções ativas entre mulheres com RSA na Jordânia.

Palavras-chave: *Toxplasma gondii, Neospora caninum,* soroprevalência, fatores de risco, aborto, Jordânia.

Introduction

Toxoplasma gondii and *Neospora* spp. are closely related obligate intracellular protozoan parasites, belonging to the phylum Apicomplexa, family Sarcocystidae. There are two known *Neospora* species namely *N. caninum* and *N. hughesi* that cause neosporosis (Dubey et al., 2007).

T. gondii is a major parasite that infects all the warm-blooded animals including Man and domestic animals, causing toxoplasmosis (Montoya & Liesenfeld, 2004). Felines are the definitive and reservoir hosts for *T. gondii*, with more infection in feral than in domestic cats (Montoya & Liesenfeld, 2004). The minimum annual losses to the USA caused by *T. gondii* infection in man is estimated at $ 392 million (Roberts et al., 1994).

Lymphadenitis is the most common manifestation of human acute toxoplasmosis (Montoya & Liesenfeld, 2004). The acute phase is followed by a subclinical chronic phase. However, congenital fetal involvement, and abortion are the most important documented results (Torgerson & Mastroiacovo, 2013).

Seroprevalences of *T. gondii*-IgG and IgM in women have been reported from all over the world. The highest seroprevalence of anti-*T. gondii*-IgG was 84.3% in Mayotte Island (Julvez et al., 1994) while one of the lowest was 4.7% in India (Mohan et al., 2002). The seroprevalences of anti-*T. gondii*-IgM varied between 20.7% in repeatedly aborted females in Mexico (Galván Ramírez et al., 1995), and 0% among the Thai women (Sukthana, 1999). Globally, the reported seroprevalences of *T. gondii*-IgG and IgM among recently spontaneously aborted (RSA) women ranges between 0% to 46.1% and 0% to 18.4 (Nayeri et al., 2020) respectively. In contrast to *T. gondii*-IgG, *T. gondii*-IgM studies in the Middle East and in Jordan are very few (Qublan et al., 2002; Nimri et al., 2004).

Many risk factors for *T. gondii*-IgG seropositivity were reported from many countries (Nayeri et al., 2020). Keeping cats inside the house was found to be either an enhancing risk factor (Baril et al., 1999; Fan et al., 2001; Bahia-Oliveira et al., 2003; Wam et al., 2016), or a factor with no effect (Bobić et al., 1998; Bobić et al., 2003; Shuhaiber et al., 2003). Concerning feeding habits; consumption of under cooked meat, eating raw vegetables and drinking raw milk are the most incriminated risk factors (Kapperud et al., 1996; Bobić et al., 1998; Paul, 1998; Nissapatorn et al., 2003). In addition, consumption of raw lamb, beef and pork are important enhancing risk factors (Bobić et al., 1998; Paul, 1998; Fan et al., 2001; Elnahas et al., 2003; Schnieder, 2003). Low educational level is an enhancing risk factor (Elnahas et al., 2003), while high educational level is a reducing factor (Nissapatorn et al., 2003). Childbearing age and pregnancy are enhancing risk factors (Avelino et al., 2003).

In northern Jordan, the risk factors for seropositivity to *T. gondii*-IgG were studied in undergraduate female university students (Obaidat et al., 2015), pregnant (Jumaian, 2005; Nimri et al., 2004) and repeatedly aborted women (Abdel-Hafez et al., 1986; Qublan et al., 2002). Age, rural residence, consumption of undercooked meat and soil contact were found to be associated with seropositivity.

N. caninum is a well-known parasite of domestic and wild animals that utilizes a wide range of animals as intermediate hosts (Khan et al., 2020). Cattle, sheep, horses, goats, foxes, deer, buffaloes, and camels are its natural intermediate hosts and, in addition, cats, mice, rats, gerbils, and monkeys are experimental intermediate hosts, and many wild animal reservoirs (Gondim, 2006). Dogs and other canids have been identified as both intermediate and definitive hosts of this parasite (Gondim et al., 2004). Dogs are likely to become infected by the ingestion of contaminated tissue, including aborted fetuses, dead animals/birds, or placentas and shed oocysts in their faces, which can serve as a major source of infection for other species. In the dog, inflammatory lesions of skeletal muscles and the central nervous system predominate (Dubey et al., 2009). *N. caninum* is considered as a significant cause of abortion in ruminants, especially cattle Anderson et al. (1991). Similarly, experimental infection of pregnant monkeys with *N. caninum* resulted in transplacental transmission of *N. caninum* (Barr et al., 1994).

Humans are exposed to two *N. caninum* infective stages; oocyst and tissue cyst. The oocysts are found in the contaminated environment, and the tissue cysts are located in food animal tissues (improperly cooked meat) and *N. caninum*-DNA was found in cow's milk, including colostrum (Milne et al., 2006; Moskwa et al., 2007). However, there is no conclusive evidence that lactogenic transmission of *N. caninum* occurs in nature (Dubey et al., 2007).

Seropositivity of *N. caninum* was reported in Man ranging from 0% to 38%. Several human populations were examined for anti-*N. caninum*-IgG, 1) healthy blood donors in Korea (Nam et al., 1998), Northern Ireland (Graham et al., 1999) and Central California, USA (Tranas et al., 1999). 2) samples submitted for microbiological and biochemical testing in the UK (McCann et al., 2008) and France (Robert-Gangneux & Klein, 2009) were tested at a titer of 1/100 and had seropositivity of 0 to 8%. 3) AIDS patients in Brazil, at a lower IFAT titer (1/50) yielded seropositivity in 38% of 61 and 18% of 50 neurological disorder patients (Lobato et al., 2006) and HIV positive subjects in France yielded 1% at 1/80 titer and in Brazil yielded 0.6% at a higher titer of 1/200 (Oshiro et al., 2015). 4) two attempts testing women with repeated abortion in Denmark (Petersen et al., 1999) and the UK (Hemphill & Gottstein, 2000), at a titer of 1/400, yielded no seropositive women. 5) Four studies employing pregnant women

from Egypt (Ibrahim et al., 2009), Senegal (Coulibaly et al., 2020) and Brazil (Duarte et al., 2020a, b) reported higher seroprevalences of 8%, 14% 23% and 24% respectively.

Recently, *N. caninum* DNA was detected in the umbilical blood of two women in Brazil (Duarte et al., 2020b) raising the concern about the zoonotic potential of *N. caninum*.

In Jordan, *N. caninum* seroprevalences have been reported in sheep, goats (Abo-Shehada & Abu-Halaweh, 2010) and cattle (Talafha & Al-Majali, 2013) and *Neospora* spp. seroprevalence has been reported in horses (Talafha et al., 2015; Abu-Halaweh et al., 2020) at high rates.

The seroprevalence of both anti-*T. gondii* and anti-*N. caninum* IgG and IgM antibodies in RSA women have not been reported in Jordan nor have the seroprevalences of those antibodies been reported anywhere in the World for *N. caninum*. This cross-sectional study compares the true seroprevalences of *T. gondii* and *N. caninum* specific IgG and IgM among RSA women in Jordan and investigates some risk factors for *T. gondii* and *N.* caninum seropositivity.

Materials and Methods

Sample size determination and sampling

As the expected seroprevalences had a wide range, a seroprevalence of 50% was used. As a 95% confidence level and a 5% absolute value precision are required according to Thrusfield (2007), the appropriate sample size is 384. During the period January to July 2003, a total of 445 blood samples were collected from RSA women, 7-10 days after abortion.

Hospitals and subjects

Three hospitals (2 public and 1 private) were employed. The two public hospitals were Al-Bashir, which is the largest public hospital in Jordan, located in Amman serving patients from central Jordan especially those of poor socio-economic standard, and Al Hussein Hospital, the fifth largest public hospital, located in Al-Salt City. Hiba is a private maternity hospital located in Amman serving mainly patients from the Christian community. The women were recruited from the outpatient clinics of the hospitals. A qualified nurse approached them and explained the objectives of the study. Of the women approached 48 did not want to have their blood drawn; in which cases other eligible women were approached and recruited.

The definition of a case of miscarriage was the spontaneous end of a pregnancy after the first 6 weeks and prior to 20 weeks of gestation. Pregnancy losses after 20 weeks are stillbirths.

Serology

Blood samples were collected, and sera were separated and stored at -20° C until testing at the end of the collection period.

Serum samples were tested for anti-*T. gondii*-IgG and IgM antibodies using Enzyme Immunoassay test kits (Biocheck, Inc, 837 Cowan Rd. Burlingame, CA 94010). According to the manufacturer, the IgG test sensitivity and specificity are 95% and 97.3% respectively and the IgM test sensitivity and specificity are 95.5% and 96.5% respectively.

Serum samples were tested for anti-*N. caninum*-IgG and IgM. Slides spotted with whole *N. caninum* (NC-1 strain) tachyzoites were purchased from VMRD, Inc., (Pullman, WA.). Cattle positive sera and anti-cattle sera labelled with fluorescein were used until positive human sera were found, after which the positive human sera were used. A cut-off titer of 1/200 was considered positive (Tranas et al., 1999; Dubey et al., 2009). The sample was considered positive when the fluorescent reactions involved all the periphery of the tachyzoites (Paré et al., 1998). The sensitivity and specificity of this test were 100% and 98% respectively when animal sera were used (Packham et al., 1998).

Data collection and ethical considerations

Consenting volunteer RSA women briefed on the objectives of the study, were interviewed using a questionnaire in Arabic which had been pilot tested. They were interviewed by a qualified nurse during the outpatient visit and at the time of blood collection. Information collected included name, type of hospital, age, place of residence, cat and dog in the house or garden/yard, feeding habits (eating raw meat, barbecued meat, or raw wild plants),

number of miscarriages and stillbirths, pregnancy age at the time of abortion. The study protocol was approved by the concerned committees at Jordan University of Science and Technology. All identifying information was kept confidential.

Data analysis

Data were stored in a database and analyzed using SPSS, version 10.0. The true seroprevalence and the 95% confidence interval (CI) were calculated according to Rogan & Gladen (1978). For both parasites true seroprevalence, the smallest sensitivity and specificity were employed. Univariate and multivariable analyses were performed. The dependent variable was parasite-Ig-seropositive status, coded as 0 (negative) or 1 (positive). A total of nine variables were tested. Screening of the significant variables to be used in the final logistic regression was conducted using univariable analysis. One variable at a time was tested for associations with *N. caninum*/*T. gondii*-seropositivity using the Chi-square test. For ordered categorical variables, the Chi-square test for trends was employed. Fishers exact test was used when the expected frequency was less than five. A multivariable analysis was then conducted starting with all factors that had a $p \leq 0.05$ or an OR ≤ 0.3 or an OR ≥ 3.0 in the univariable analysis. Only those factors that remained in the final models were presented. A p-value of < 0.05 was considered statistically significant. Odds ratios and its 95% confidence intervals were calculated.

Results

RSA women were aged between 15 and 45 years with quartiles of: Q1 = 24 years, Q2 = 27 years and Q3 = 32 years.

Table 1 summarizes the true seroprevalences results. The true seroprevalence of *N. caninum*-IgM was significantly ($p < 0.05$) higher than that of *T. gondii*-IgM. Figure 1 summarizes the number of *N. caninum*-IgG and IgM positive sera and their titers up to 1/1000.

Tables 2 and 3 summarize the univariate analysis results. Two variables, eating barbecued meat and number of abortions were associated with *T. gondii*-IgM seropositivity with both univariate and logistic regression analyses (Table 2 and 4) and none of the studied variables were associated with *T. gond*ii-IgG seropositivity with both

Table 1. Apparent and true seroprevalences of *Toxoplasma gondii* and *Neospora caninum* among 445 recently spontaneously aborted women in Jordan

Ig class	No. +ve	Seroprevalence % (95% CI)	
		Apparent	True
***T. gondii* (ELISA)**			
G	133	29.9 (25.7, 34.2)	22.1 (18.2, 26.0)
M	26	5.8 (03.6, 08.0)	2.6 (01.1, 04.0)
G&M	9	2.0 (0.1, 3.3)	0.0 (0, 0)
***Neospora caninum* (IFAT, at a titer of 1:200)**			
G	110	24.7 (20.7, 28.7)	22.7 (18.8, 26.6)
M	55*	12.4 (09.3, 15.5)	10.6 (07.7, 13.4)
G&M	39	8.8 (06.2, 11.4)	6.7 (04.4, 09.0)
Both parasites			
G	31	7.0 (04.6, 09.4)	04.6 (02.7, 06.6)
M	0	0.0 (0, 0)	0.0 (0, 0)
G&M	0	0.0 (0, 0)	0.0 (0, 0)

*Significantly higher than that of *T. gondii* (X^2 = 11.4, df = 1, p = 0.007).
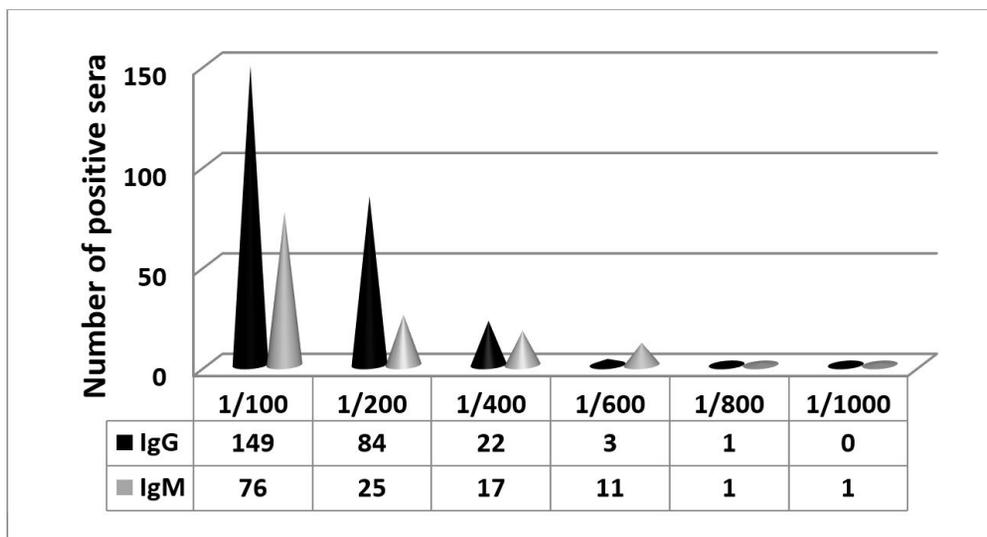
Figure 1. Number of positive sera with anti-*Neospora caninum*-IgG and IgM titers among 445 recently spontaneously aborted women in Jordan.

Table 2. Univariate association between risk factors and *Toxoplasma gondi*-IgG and IgM seropositivity among 445 recently spontaneously aborted women in Jordan

Variable	Category	No.	No. IgG +ve (%) (n = 133)	p	No. IgM +ve (%) (n = 27)	p
Type of hospital	Public	410	121 (30)	0.985	26 (6)	0.407
	Private	35	12 (34)		1 (3)	
Age group	15-20	15	4 (27)	0.767	1 (5)	0.127
	21-25	169	47 (28)		6 (4)	
	26-30	131	40 (31)		7 (5)	
	31-35	69	22 (32)		5 (7)	
	36-40	57	18 (32)		8 (14)	
	41-45	4	2 (50)		0 (0)	
Cats in house	No	42	14 (33)	0.346	2 (5)	0.503
	Yes	81	20 (25)		3 (4)	
	Garden/yard	300	99 (33)		22 (7)	
Dogs in house	No	338	106 (31)	0.677	21 (6)	0.797
	Yes	29	11 (38)		2 (7)	
	Garden/yard	56	16 (29)		4 (7)	
Eating raw meat	No	417	123 (30)	0.518	24 (6)	0.237
	Yes	28	10 (36)		3 (11)	
Eating Barbecued meat	No	9	3 (33)	1.000	3 (33)	0.013
	Yes	436	130 (30)		24 (6)	
Eating wild plants	No	294	90 (31)	0.664	19 (7)	0.626
	Yes	151	43 (29)		8 (5)	
No. of abortions	1	149	47 (32)	0.337	4 (3)	0.001
	2	215	62 (29)		14 (7)	
	3	69	18 (26)		3 (4)	
	>4	12	6 (50)		6 (50)	
Stillbirth	No	422	129 (31)	0.239	24 (6)	0.156
	Yes	23	4 (17)		3 (13)	

Table 3. Univariate association between risk factors and *Neospora caninum*–IgG and IgM seropositivity among 445 recently spontaneously aborted women in Jordan.

Variable	Category	No.	No. IgG +ve (%) (n = 110)	p	No. IgM +ve (%) (n = 55)	p
Type of hospital	Public	410	101 (25)	0.841	45 (11)	0.012
	Private	35	09 (26)		10 (29)	
Age group	15-20	15	5 (33)	0.018	2 (13)	0.302
	21-25	169	35 (21)		19 (11)	
	26-30	131	25 (19)		12 (9)	
	31-35	69	25 (36)		10 (15)	
	36-40	57	20 (35)		12 (21)	
	41-45	4	0 (0)		0 (0)	
Cats in house	No	44	13 (30)	0.104	4 (9)	0.823
	Yes	87	14 (16)		10 (12)	
	Garden/yard	314	83 (26)		41 (13)	
Dogs in house	No	358	71 (20)	0.001	40 (11)	0.235
	Yes	29	10 (34)		5 (17)	
	Garden/yard	58	29 (50)		10 (17)	
Eating raw meat	No	417	101 (24)	0.347	49 (12)	0.139
	Yes	28	9 (32)		6 (21)	
Eating Barbecued meat	No	9	3 (33)	0.696	0 (0)	0.605
	Yes	436	107 (25)		55 (13)	
Eating wild plants	No	294	73 (25)	0.940	41 (14)	0.156
	Yes	151	37 (25)		14 (9)	
No. of abortions	1	149	39 (26)	0.538	22 (15)	0.214
	2	215	47 (22)		20 (9)	
	3	69	19 (28)		10 (15)	
	>4	12	5 (42)		3 (33)	
Stillbirth	No	422	109 (26)	0.022	54 (13)	0.337
	Yes	23	1 (4)		1 (4)	

Table 4. Multivariate logistic regression models of factors associated with seropositivity to *Toxoplasma gondii*-IgM (ELISA) and *Neospora caninum*-IgG and IgM (IFAT at a cutoff titer 1/200) among 445 recently spontaneously aborted women in Jordan

Protozoal-Ig class	Risk factor	Category	p	OR	95% CI
***T. gondii*-M**[a]	Number of abortions	>4	0.001	2.4	1.5, 3.7
	Eating barbecued meat	Yes	0.012	0.1	0.02, 0.6
***N. caninum*-G**[b]	Dog (s) contact	In The house	0.001	2.1	1.5, 2.7
	Had a stillbirth	Yes	0.038	0.1	0.02, 0.9
***N. caninum*-M**[c]	Type of hospital	Private	0.016	2.7	1.2, 6.2

[a]Likelihood ratio of chi-square (LR $_x2$) = 23.25 on 2 degrees of freedom, $p = 0.001$, Adjusted R-Squared (AR^2) = 0.072; [b](LR $_x2$) = 31.4 on 2 degrees of freedom, $p = 0.001$, AR^2 = 0.068; [c]LR $_x2$ = 5.1 on 1 degrees of freedom, $p = 0.024$, AR^2 = 0.011.

univariate and logistic regression analyses. Three variables, namely, age group, dog contact and had a stillbirth were associated with *N. caninum*-IgG seropositivity with univariate analysis (Table 3). After stepwise selection, the final model had dog contact in the house and had a stillbirth (Table 4). Only one variable, the type of hospital, was associated with *N. caninum*-IgM-seropositivity with both univariate and logistic regression analyses (Table 3 and 4).

Discussion

The current results showed a significantly ($p < 0.05$) higher true seroprevalence of *N. caninum*-IgM compared to *T. gondii*-IgM but similar with the respect of IgG against both parasites. A portion of the sample had only *N. caninum*-IgM antibodies, a second had both IgM and IgG and a third had IgG only (Table 1), which is suggestive of active infections at different stages during pregnancy in RSA women.

Only 7.0% (95% CI: 4.6, 9.4) of women had both anti-*T. gondii*-IgG and anti-*N. caninum* IgG (Table 1), which is similar to previous findings of 5.9% and 8.4% seropositivity among pregnant women in Egypt (Ibrahim et al., 2009) and Brazil (Duarte et al., 2020b) respectively.

In northern Jordan, the apparent seroprevalence of *T. gondii*-IgG is 57% in repeatedly aborted women (Abdel-Hafez et al., 1986) was similar to the 54% finding in high-risk women who had abnormal pregnancies (Nimri et al., 2004). However, the current results showed lower apparent seroprevalence among RSA women (Table 1).

Analogous with the current results (Table 1), the seropositivity of *T. gondii*-IgM in pregnant women in northern Jordan was reported at 1.5% (2 of 132) (Qublan et al., 2002) and in Central Jordan at 2.7% (4 of 148) in high-risk women who had abnormal pregnancies (Nimri et al., 2004).

Most of previous *N. caninum* seroprevalence studies employed IFAT and reported the results at different titers, which impacts the validity of the seropositivity comparison. A cutoff titer of 1/50 in the IFAT was considered fitting to avoid cross-reactivity between *N. caninum and T. gondii* in animal studies (Lobato et al., 2006; Silva et al., 2007). A cutoff titer of 1/160 for IFAT was found to give the greatest sensitivity and specificity values for *N. caninum* (Packham et al., 1998) in cattle and a titer of 1/50 was recommended for dogs (Dubey et al., 2009). For this study, a cut-off titer of 1/200 was considered positive (Tranas et al., 1999).

By contrast, the search for anti-*N. caninum*-IgM took two attempts using IFAT. One yielded no seropositivity in normally delivered umbilical cord blood (Duarte et al., 2020b) and another in pregnant women reported 5.9% seropositivity at a 1/100 titer (Duarte et al., 2020a).

The reported high seroprevalences in Jordan is a reflection of the heavy environmental contamination with the infective stages, as well as the vertical transmission of the parasite in livestock (Dubey et al., 2007) which has been proved experimentally in nonhuman primates (Barr et al., 1994) but not yet in women.

However, European studies from Denmark (Petersen et al., 1999) and the UK were negative for the *N. caninum* test (Hemphill & Gottstein, 2000). The low *N. caninum* seropositivity in Europe may be explained by differences with other countries reporting higher seropositivity rates. For example, an association between *N. caninum* seropositivity and climate was found in livestock, in Europe (Julvez et al., 1994; Rinaldi et al., 2005). In Jordan, which encompasses six climatic zones and a high seroprevalence of *Neospora* spp., only the cool temperate rainy climatic zone was associated with reduced small ruminant flock-level seroprevalence of *N. caninum* (Abo-Shehada & Abu-Halaweh, 2010) and with horse titers <1/400 (Abu-Halaweh et al., 2020).

In addition, seroprevalences of parasites transmitted from dog to Man are influenced by locally adopted measures aimed at controlling stray dogs, reducing parasitic infections in owned dogs, and mitigating environmental contamination with the parasites. Such measures included, dog testing and treatment, the use of parasite free and cooked dog feed and proper canine waste disposal. Each of those measures play a role in mitigating the spread of infection in Man and animals and are reflected in the frequencies of parasites transmitted from dog to Man, including *N. caninum.*

Globally, most previous studies concentrated on consumption of raw/undercooked meat as an enhancing risk factor for *T. gondii* seropositivity. None studied properly cooked meat as an important food, representing a source of needed nutrients for the pregnant woman, and very few studies investigated risk factors for *T. gondii*-IgM seropositivity.

As consuming blood is prohibited in Islam, most Jordanians eat well cooked meat and only 6% (28 of 445) of the examined women ate raw or undercooked meat. This feeding habit is expected to reduce the transmission of the studied parasites through eating meat. In Jordan, eating barbecued meat reflects high socio-economic status

and can be confounded with malnutrition and education level. On the other hand, well cooked meat provides nutrients needed by pregnant women and its consumption is associated with reduced *T. gondii*-IgM seropositivity. The association between nutritional status and resistance to protozoal parasites is well established and reported with regards to other protozoal infections. For example, malnutrition was reported as a risk factor for severe visceral leishmaniasis (Cerf et al., 1987). This finding emphasizes the need of pregnant women for good balanced nutrition to safeguard their own health and that of their unborn children.

To date, as far as is known, only three risk factors for *N. caninum*-IgG seropositivity in pregnant women were reported. These are: eating Senegalese barbecued meat (OR = 3.5) (Coulibaly et al., 2020) and the presence of domestic animals (OR = 2.3), and dogs (OR = 2.0) [11] (Duarte et al., 2020b).

Epidemiological evidence for the role of the dog in neosporosis, was found before the discovery of the dog as a definitive host for this parasite. This was an association between seropositivity of *N. caninum* in cattle and the presence of dogs (Bartels et al., 1999). As with *N. caninum* seropositivity in livestock hosts, e.g., cattle and small ruminants (Abo-Shehada & Abu-Halaweh, 2010), and the cord blood of women (Duarte et al., 2020b), *N. caninum*-IgG seropositivity in RSA women was associated with the presence of dogs in the house (OR = 2.6).

N. caninum-IgG seropositivity is associated negatively (OR= 0.1) with fetal loss during the second half of pregnancy. A significant inhibitory effect on the intracellular multiplication of the *N. caninum* in cells was demonstrated *in vitro* using sheep serum which had anti-*N. caninum* antibodies (Omata et al., 2005). *N. caninum*-IgM antibodies are detectable within two weeks of infection, while *N. caninum*-IgG requires several weeks, peaking six months later (Khan et al., 2020).

N. caninum-IgM seropositivity was associated with the type of hospital visited, as the women who used the private hospital had high odds of *N. caninum* seropositivity. The private hospital included in this work was utilized by Christian women, and women of a high socio-economic standard who could afford the private hospital costs and may have closer contact with pet dogs unlike those of the Muslim population in Jordan by whom dogs are perceived as unclean. Additionally, the low number of hospitals tested may have biased the results.

Conclusions

Jordanian RSA women are equally exposed to both *N. caninum* and *T. gondii* with a significantly ($p < 0.05$) higher true seroprevalence of *N. caninum*-IgM compared to that of *T. gondii,* which is suggestive of active infections. Despite sero-evidence of *N. caninum*-IgG and IgM and molecular evidence in the blood of two human umbilical cords, *N. caninum* has yet to be isolated from human tissue. The zoonotic potential and the role of *N. caninum* in human pregnancy needs to be investigated.

The current findings suggest that the aborted human placenta is a suitable tissue for isolating *N. caninum* from Man and bring the zoonosis verdict closer than ever. The causal relationship between *N. caninum* infection and spontaneous abortion can be explored by utilizing a large cohort study of pregnant women infected with *N. caninum* compared to controls. An avidity ELISA was developed to discriminate between acute and chronic antibody titers in individual cattle (Björkman et al., 1999). There is a need to develop a similar assay for use in humans.

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