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# FACTORS ASSOCIATED TO INFECTION BY *Toxoplasma gondii* IN PREGNANT WOMEN ATTENDED IN BASIC HEALTH UNITS IN THE CITY OF ROLÂNDIA, PARANÁ, BRAZIL

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### **SUMMARY**

The aim of the present work was to determine the prevalence of IgG and IgM anti-*Toxoplasma gondii* antibodies and the factors associated to the infection in pregnant women attended in Basic Health Units in Rolândia, Paraná, Brazil. The sample was divided in two groups: group I (320 pregnant women who were analyzed from July 2007 to February 2008) and group II (287 pregnant women who were analyzed from March to October 2008). In group I, it was found 53.1% of pregnant women with IgG reactive and IgM non-reactive, 1.9% with IgG and IgM reactive, 0.3% with IgG non-reactive and IgM reactive and 44.7% with IgG and IgM non-reactive. In group II, it was found 55.1% with IgG reactive and IgM non-reactive and 44.9% with IgG and IgM non-reactive. The variables associated to the presence of IgG antibodies were: residence in rural areas, pregnant women between 35-40 years old, low educational level, low family income, more than one pregnancy, drinking water which does not originate from the public water supply system and the habit of handling soil or sand. Guidance on primary prevention measures and the quarterly serological monitoring of the pregnant women in the risk group are important measures to prevent congenital toxoplasmosis.

KEYWORDS: Toxoplasmosis; Risk factors; Diagnostic; Congenital disease.

## INTRODUCTION

Toxoplasmosis is a worldwide infection caused by *Toxoplasma gondii*<sup>46</sup>, an obligatory intracellular parasite that infects warm-blooded animals, including human beings<sup>30</sup>. It is believed that up to one third of the world's population is infected<sup>21</sup>. The prevalence of infection by *T. gondii* in humans varies among different countries, geographical regions within a country, social and economic strata and cultural habits<sup>57</sup>.

Countries with a high level of toxoplasmosis have instituted and obtained success in secondary prevention programs by screening maternal serum<sup>26</sup>. In the acute infection, the levels of immunoglobulin G (IgG) and immunoglobulin M (IgM) increase between one and two weeks of infection<sup>44</sup>. Positive IgM results should be confirmed in a reference laboratory<sup>60</sup> with capability to delimit the infection period by using specific tests such as the IgG avidity test<sup>31</sup>.

For occurrence of congenital toxoplasmosis, the primary infection should be established during pregnancy  $^{16}$ . Among women infected by T. *gondii* during pregnancy, 61% do not transmit the disease to the fetus, 26% of the conceptions present subclinical infection (visual/or hearing deficit, neuromotor and/or learning deficit) and in 13% there is a clinical infection (hydrocephalus or microcephaly, chorioretinitis, cerebral calcification and mental retardation) $^{43}$ .

The importance of implanting maternal serum screening to detect toxoplasmosis lies in the possibility of adopting prophylactic and therapeutic measures to minimize the vertical transmission and/or decrease the damage to fetus development<sup>15</sup>. In the face of the seriousness of the congenital disease, it is fundamental to start prenatal care in the first three months of pregnancy, so that, acute cases of congenital toxoplasmosis can be early identified<sup>40</sup>.

The aim of the present study was to assess the factors associated to infection by *T. gondii* and determine the prevalence of IgG and IgM anti-*T. gondii* antibodies in pregnant women attended in Basic Health Units (BHU) of Rolândia, Paraná, during the implementation of the "Surveillance Program of Toxoplasmosis Acquired in Pregnancy and Congenital".

## MATERIAL AND METHODS

A cross-sectional study was carried out in pregnant women who received prenatal care in the six BHU in the city of Rolândia, Paraná, with an average of 750 per year attended by the Brazilian Public Health Service. The city is located in the north of Paraná State, the metropolitan region of Londrina, and has a population of 56,352 inhabitants<sup>52</sup>.

In the study, all the pregnant women who entered for the first prenatal visit in the BHU were evaluated and filled out a term of free and informed

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consent and an epidemiological questionnaire. The prenatal screening program was set up in July 2007. Before the program implementation, the serological test for toxoplasmosis was not included in the pre-natal routine. In conjunction with the State University of Londrina and the Municipal Health Secretariat of Rolândia, Paraná, the program actions were established according to the characteristics of the health service of the city.

In March, 2009, the serological exams for toxoplasmosis were carried out by a private laboratory contracted by the Municipal Health Secretariat of Rolândia, Paraná, and was oriented to determine a new serological test based on the "Acquired Toxoplasmosis in Pregnancy and Congenital: Health Surveillance, Diagnosis, Treatment and Conduct" Thus the Indirect Immunofluorescence Assay (IFA) and the Enzyme-Linked Immunosorbent Assay (ELISA) were used to research IgG anti-*T. gondii* antibodies, as they had a good correlation 14.58.

Based on the change in technique to detect IgG and IgM anti-*T. gondii* antibodies, the pregnant women were divided into two groups and two phases:

**Group I (G I) - First phase of the study:** In this group, pregnant women were attended from July 2007 to February 2008. The research of IgG anti-*T. gondii* was performed by Indirect Immunofluorescence Assay (IFA), with a cut off point of 1:16<sup>13</sup> and the research of IgM antibodies was performed by Microparticle Enzyme Immunoassay (MEIA) (Abbott Laboratories, Illinois, USA). The serological tests were carried out at the Immunology Laboratory of the University Hospital of Londrina.

Group II (G II) - Second phase of the study: In this group, pregnant women were attended from March to October 2008. The research of IgG anti-*T. gondii* was performed by the ELISA technique, with a cut off point of 15.0 UI/mL and the research of IgM antibodies was performed by Capture ELISA, with a cut off point 1.1 UI/mL, both from DiaSorin® (DIASORIN, Saluggia, Italy). The serological tests were carried out in a private contracted laboratory.

The reactive IgG and IgM samples, in both groups, were assessed by the IgG avidity test LIAISON® (DIASORIN, Saluggia, Italy) to detect possible acute infection.

The present study was approved by the Ethics Committee for Research in Human Beings at the State University of Londrina, document number 182/07 and was carried out in conjunction with the Municipal Secretariat of Health of Rolândia, Paraná.

### RESULTS

Table 1 shows the prevalence of IgG and IgM in pregnant women in G I and G II, and the general prevalence presented. The analysis of proportions showed that there was no prevalence difference between the groups, that is, the infection rate in both tests were similar (Table 1).

In G I, all the six sera samples IgG and IgM reactive were submitted to the IgG avidity test and they showed strong results. The pregnant women who had non-reactive IgG but reactive IgM were submitted to a new serology test 15 days later and the same result was obtained.

Among 176 pregnant women with reactive IgG, 35 (19.9%) presented titters of 16, 73 (41.5%) of 64, 49 (27.8%) of 256, 17 (9.7%) of 1024 and two (1.1%) of 4000.

Tables 2 and 3 show the factors associated to the presence of IgG anti-T. gondii antibodies which were: residence in rural area (p = 0.0140), pregnant women between 35 and 40 years (p = 0.0043), low educational level ( $\leq 8$  years of study) (p = < 0.0001), monthly family income less than one minimum wage (p = 0.0220), more than one pregnancy (p = 0.0116), drinking water which does not originate from the public water supply system (p = 0.0011), the habit of handling soil or sand (p = 0.0497) and a tendency (p = 0.0524) in the habit of handling raw meat.

# DISCUSSION

This was the first epidemiological study carried out with pregnant women in the city of Rolândia, Paraná, Brazil, in which the prevalence of IgG anti-*T. gondii* antibodies found was 55.0% and 55.1% in groups I and II, respectively, and the general prevalence was 55%. The prevalence detected for IgM anti-*T. gondii* antibodies was 2.2%.

The prevalence of IgG anti-T. gondii antibodies was similar to the

 Table 1

 Prevalence of IgG and IgM anti-Toxoplasma gondii antibodies in 670 pregnant women attended in Basic Health Units in Rolândia, Paraná, 2007-2008

Antibodies	Prevalence/total (%)		p value	General prevalence/total (%)
	Group 1	Group 2		
Seropositivity				
IgG (+); IgM (-)	170/320 (53.1)	158/287 (55.1)	-	<b>IgG</b> (+)
IgG (+); IgM (+)	6/320 (1.9)	0	0.6934	
Total	176/320 (55.0)	158/287 (55.1)		334/607 (55)
Susceptibility				
IgG (-); IgM (-)	143/320 (44.7)	129/287 (44.9)	-	<b>IgG</b> (-)
IgG (-); IgM (+)	1/320 (0.3)	0	0.9861	
Total	144/320 (45.0)	129/287 (44.9)		273/607 (45)

(+) reactive; (-) non-reactive.

Table 2

Analysis of the socioeconomic variables associated to the presence of IgG anti-Toxoplasma gondii antibodies in pregnant women attended in Basic Health Units in Rolândia, Paraná, 2007-2008

Variables	Reactive IgG/total (%)	p	Odds ratio (CI 95%)
Residence			
Rural area	28/37 (75.68)	0.0140	2.70 (1.19 - 6.29)
Urban area	305/570 (53.51)		
Age group (years)			
15 - 24 *	137/276 (49.64)		
25 - 35	151/267 (56.55)	0.1263	1.32 (0.93 - 1.88)
36 - 45	45/64 (70.31)	0.0043	2.40 (1.29 - 4.51)
Educational level			
≤ 8 years of school	85/124 (68.55)	0.0008	2.07 (1.34 - 3.22)
> 8 years of school	247/482 (51.24)		
Monthly family income**			
less than one M.W.*	32/42 (76.19)		
From 1 to 3 M.W.	247/436 (56.65)	0.0220	0.41 (0.18 - 0.89)
More than 3 M.W.	52/101 (51.49)	0.0108	0.33 (0.14 - 0.80)
Number of pregnancies			
One pregnancy	118/267 (44.19)	0.0116	0.69 (0.52 - 0.92)
More than one pregnancy	215/338 (63.61)		
Drinking water does not originate from the public			
water supply system			
Yes	304/571 (53.24)	0.0011	0.24 (0.09 - 0.61)
No	29/35 (82.86)		

<sup>\*</sup>Reference category; \*\* M.W. = Minimum wage current (R\$ 380,00 until march 2008, R\$ 415,00 after march 2008).

Table 3

Analysis of the alimentary and hygiene habits and behavior variables associated to the presence of IgG anti-*Toxoplasma gondii* antibodies in pregnant women attended in Basic Health Units in Rolândia, Paraná, 2007-2008

Variables	Reactive IgG/total (%)	p	Odds ratio (CI 95%)
Presence of vegetable garden at home			
Yes	60/99 (60.60)	0.2520	1.32 (0.83 - 2.10)
No	273/508 (53.74)		
Contact with cats			
Yes	30/63 (47.62)	0.2773	0.72 (0.42 - 1.26)
No	303/544 (55.70)		
Habit of consumption of raw or poorly cooked meat			
Yes	74/128 (57.81)	0.6145	1.13 (0.75 - 1.71)
No	256/467 (54.82)		
Habit of handling soil or sand			
Yes	43/64 (67.18)	0.0497	1.79 (1.00 - 3.21)
No	290/543 (53.41)		
Habit of eating raw vegetables			
Yes	277/491 (56.42)	0.1386	1.39 (0.91 - 2.12)
No	56/116 (48.27)		
Habit of handling raw meat			
Yes	289/510 (56.66)	0.0524	1.58 (1.00 - 2.49 )
No	44/97 (45.36)		

findings in studies carried out in pregnant women in other states of Brazil, such as the 54.8% prevalence found in Pelotas, Rio Grande do Sul<sup>12</sup>, 57.3% in São José do Rio Preto, São Paulo<sup>27</sup>, 59.8%<sup>59</sup> and 61.1%<sup>49</sup> both in Porto Alegre, Rio Grande do Sul State. In the city of Londrina, also in the north of the of Paraná State, LOPES *et. al.* (2009)<sup>38</sup> and MITSUKA-BREGANÓ (2009)<sup>41</sup> detected 49.2% and 50.5% reactive sera for IgG, respectively. Higher values were reported in other regions, such as: 71.5% in Fortaleza, Ceará State<sup>51</sup>, 73.5% in Vitória, Espírito Santo State<sup>5</sup> and 74.5% in the region of Alto Uruguai, northwest of Rio Grande do Sul <sup>56</sup>.

The Brazilian data are higher when compared to other countries: 9.1% in the United Kindgom<sup>45</sup>, 10.6% in China<sup>37</sup>, 10.9% in Norway<sup>32</sup>, 28.6% in Spain<sup>9</sup>, 29.4% in Greece<sup>3</sup> and 30.1% in Turkey<sup>25</sup>. The prevalence of seropositive individuals for *T. gondii* varies greatly among the different regions of the world. Several factors can interfere, such as climate, age, hygiene, alimentary and cultural habits, socioeconomic strata and other habits that can result in greater contact with *T. gondii*<sup>33,59</sup>.

Regarding the prevalence of IgM antibodies, MITSUKA-BREGANÓ (2009)<sup>41</sup> and LOPES *et al.* (2009)<sup>38</sup> found a lower prevalence in pregnant women in Londrina, Paraná, which was 0.5% and 1.2%, respectively. Similar results were found in pregnant women such as 2.2%<sup>6</sup> in Santo Ângelo and 2.6%<sup>49</sup> in Porto Alegre, both from the Rio Grande do Sul State.

The pregnant women resident in rural areas presented a prevalence of 75.68% (28/37) and a greater chance of infection by *T. gondii* (OR = 2.70) when compared to the others from the urban areas (Table 2). SPALDING *et al.* (2005)<sup>55</sup> analyzed pregnant women in 29 cities in the region of Alto Uruguai, Rio Grande do Sul, and also detected a greater prevalence in the rural region, which was 78% compared to 71.3% in the urban region. MANDAI *et al.* (2007)<sup>39</sup> in the city of Londrina, Paraná reported a prevalence of 68.9% in the rural region and 55.7% in the urban region. LIU *et al.* (2009)<sup>37</sup> also observed a greater chance of infection by *T. gondii* in pregnant women in rural areas in the northeast of China.

Regarding age group, the pregnant women aged from 36 to 45 presented a greater chance of infection by T. gondii (OR = 2.40). This data lines up with studies carried out by LOPES et al. (2009)38 in the city of Londrina, Paraná, where they observed that the prevalence of specific IgG antibodies increased with ages between 31 to 48. In a study performed in Sergipe State with 9,500 pregnant women, a greater prevalence was found of anti-T. gondii antibodies proportionally to the age<sup>1</sup>. LEÃO et al. (2004)<sup>34</sup> analyzed pregnant woman from two maternities in Cuiabá, Mato Grosso do Sul State, and found a significant association between age and seropositivity. CADEMARTORI et al. (2008)<sup>12</sup> assessed 425 pregnant women attended in Basic Health Units in Pelotas, Rio Grande do Sul, and reported that as the age group increased, the seroprevalence also increased. SPALDING et al. (2005)<sup>55</sup> analyzed 2,126 pregnant women from 29 cities in Rio Grande do Sul State, who were attended by the Brazilian Public Health Service, and observed that the prevalence of infection increases in older age groups. DETANICO & BASSO (2006)<sup>19</sup>, in the city of Caxias do Sul, Rio Grande do Sul, also reported a significant association between prevalence and the age of women studied (p < 0.001) with greater frequency in the ones between 37 to 49 years old (51.7%). HUNG et al. (2007)<sup>29</sup> observed

in the Democratic Republic of São Tomé and Príncipe that the IgG prevalence increased significantly (85.7%) in pregnant women over 35 years old. This association can be explained by longer exposure time to the infective agent and reinforces the importance of routine serological screening in pregnant women.

Pregnant women with monthly family income less than one minimum wage (less than 380/415 BRL) and with a low educational level ( $\leq$  eight years of study) also showed greater chances of infection by T. gondii. Similar results were reported according to studies by AVELINO et al. (2004)<sup>6</sup> in pregnant women in Goiânia, Goiás State, and LOPES et al. (2009)<sup>38</sup> and MITSUKA-BREGANÓ (2009)<sup>41</sup> both in the city of Londrina, Paraná State. VARELLA et al. (2003)<sup>59</sup> in Porto Alegre, Rio Grande do Sul, noticed that maternal school education, especially of more than nine years of study, presented a clear protecting effect for anti-T. gondii seropositivity ( $p \leq 0.001$ ) and LIU et al. (2009)<sup>37</sup> in China noticed that pregnant women who did not attend school were more likely to be infected by T. gondii than those who did. These results show the importance of social, economic and educational investments as preventive methods against the diseases.

Another variable associated to infection by T. gondii was the number of pregnancies. Women who had been pregnant more than once presented a greater chance of infection by T. gondii (p = 0.0116). This result was similar to that observed by MITSUKA-BREGANÓ (2009)<sup>41</sup> in Londrina, Paraná, and BIRGISDÓTTIR et al. (2006)10 in Sweden. AVELINO et al. (2004)6, in Goiânia, Goiás, carried out a cohort study with women at fertile age and observed that the women previously pregnant were 1.7 times more likely of acquiring the infection than those who had never been pregnant before. It can be explained by the capacity of the mother's immune response and the gestational period in which the woman is, and the virulence of the parasite strain<sup>20</sup>. The maternal-fetal transmission and the severity of sequelae are related to gestational age when maternal infection occurs<sup>24</sup>. When the seroconversion occurs before 15 weeks of gestation, the rate of transmission to the fetus can be less than 5%, nevertheless, the illness severity in newborns is higher. However, if the infection occurs late in pregnancy, near term, infection rates can reach 80%, though the clinical manifestations are less severe<sup>50</sup>. Relationships between genotype and human disease certainly exist, but are still difficult to assess due to the role of host immune status and genetic background on the control of infection, and of other parasitic factors such as the infecting dose or parasite stage<sup>17</sup>. The severity of infection can be related with the parasite genotype, which describes three ancestral lineages of T. gondii and other recombinant and atypical strains<sup>48,53</sup>, and can be also related with the genetic susceptibility of the host (immune response, frequency of alleles of Major Histocompatibility Complex)<sup>28</sup>. SOUZA (2009)<sup>54</sup> genotyped 76 samples of amniotic fluid from pregnant women living in São Paulo State with serologic screening showing seroconversion and found 71.1% with the genotype II. As the genotypes I and III are considered, respectively, with high and intermediate pathogenicity, which often lead to miscarriages and premature births, could be underrepresented in studies that analyze pregnancies that reach the second and third quarterly periods, just like the case of the pregnant women included in this work<sup>54</sup>.

The drinking water which does not originate from the public water supply system implied a greater chance of infection by *T. gondii* (p = 0.0011). A similar result was reported by GALISTEU *et al.*  $(2007)^{27}$ , in

the city of São José do Rio Preto, São Paulo. In 1995, a major outbreak occurred in Canada with 100 individuals infected with toxoplasmosis due to consumption of untreated water contaminated by felid oocytes<sup>4,11</sup>. The largest world outbreak occurred in 2001 in Santa Isabel do Ivaí, Paraná, Brazil, where the municipal water reservoir was contaminated by oocytes from feces of young cats that lived in the location. 426 presented acute *T. gondii* infection and seven cases occurred in pregnant women. One had a spontaneous miscarriage and six had infected children, one of them with a serious congenital anomaly that resulted in death<sup>2</sup>. At present there are no specific regulations or approved methods to control *T. gondii* oocysts if present in public water supplies, and research in this area has been limited<sup>35,36</sup>.

The habit of handling soil or sand should also be considered in *T. gondii* infection. SPALDING *et al.* (2005)<sup>55</sup> assessed pregnant women in the region of Alto Uruguai, Rio Grande do Sul, and verified that contact with the soil was the most important factor analyzed in the study. NASH *et al.* (2005)<sup>45</sup>, in a study with pregnant women in Kent in the United Kingdom, did not find an association between infection and gardening but observed lower prevalence in the group that used gloves when handling the soil. It occurs due to the contamination of the soil by felid oocytes deposition in the feces of infected felines, that under ideal moisture and temperature conditions and in a favorable shady location can remain infected for 12 to 24 months<sup>22</sup>. On the other hand, BARBOSA *et al.* (2009)<sup>8</sup> assessed pregnant women in Natal, Rio Grande do Norte, northeast of Brazil, and did not find an association between toxoplasmosis and gardening probably because of the climate of the region, which is dry and hot throughout the year.

The habit of handling raw meat, even at the limit of statistical significance (p = 0.0524), also offers the chance of infection by T. gondii. Studies have shown the importance of this factor, such as CADEMARTORI et al.  $(2008)^{12}$  who carried out a study with pregnant women in Pelotas, Rio Grande do Sul, and found high positive rates among those who handled raw meat. KAPPERUD et al.  $(1996)^{33}$  carried out a case-control study in Norway and related the low frequency in washing kitchen knives after preparing raw meat and before preparing another food to the risk of infection by T. gondii during pregnancy.

In the present study, the consumption of raw or poorly cooked meat was not associated with infection by *T. gondii*, in spite of it being known as an important means of transmission. Similarly, PORTO *et al.* (2008)<sup>47</sup> in Recife, Pernambuco State, and ERTUG *et al.* (2005)<sup>25</sup> in Turkey did not find an association between seropositivity to *T. gondii* and raw meat ingestion.

Contact with cats was also not associated to the chance of infection by *T. gondii* as reported in the studies by NASH *et al.* (2005)<sup>45</sup>, PORTO *et al.* (2008)<sup>47</sup> and CADEMARTORI *et al.* (2008)<sup>12</sup>. In contrast, LOPES *et al.* (2009)<sup>38</sup> and AVELINO *et al.* (2004)<sup>6</sup> obtained an association to the presence of cats. In relation to living with cats, the possibility of parasite transmission by the act of touching is minimal or nonexistent. The risk of infection might exist when there is contact with the feces of cats that are eliminating oocytes after the primary infection, and the feces of these animals could remain in the environment for at least 24 hours so that the oocytes sporulate and become infectant<sup>22</sup>.

The results observed in the present study led to the conclusion that

seroepidemiological screening for toxoplasmosis in pregnant women should be adopted as routine and accompanied with orientations and counseling in the doctor's appointments so that educational practices and preventive measures can be adopted to prevent food contamination and thus prevent infection during pregnancy.

The IgG anti-*T. gondii* antibodies prevalence in the city of Rolândia was 55% and the factors associated to infection were residence in the rural area, pregnant women between 35 and 40 years of age, low educational level, monthly family income less than one minimum wage, more than one pregnancy, drinking water which does not originate from the public water supply system and the habit of handling soil or sand. Most of the factors associated to infection for *T. gondii* were relative to the social and demographic aspects that highlights the importance and need for investment in education and clean water supply for the population. It means that guidance on primary prevention measures and quarterly serological monitoring of the pregnant women in the risk group (seronegative) are important measures to prevent congenital toxoplasmosis.

The studies and evaluations conducted during the deployment of "Surveillance Program of Toxoplasmosis Acquired in Pregnancy and Congenital" in the city of Rolândia, Paraná were fundamental and strategical to order the actions, in the training of the health professionals, in establishing the routine for diagnosis and monitoring during pregnancy and in the toxoplasmosis notification.

## **RESUMO**

## Fatores associados à infecção por *Toxoplasma gondii* em gestantes atendidas nas Unidades Básicas de Saúde do Município de Rolândia, Paraná, Brasil

O objetivo deste trabalho foi determinar a prevalência de anticorpos IgG e IgM anti-Toxoplasma gondii e os fatores associados à infecção em gestantes atendidas nas Unidades Básicas de Saúde de Rolândia. Paraná, Brasil. A amostra foi dividida em dois grupos: grupo I (320 gestantes, analisadas entre julho de 2007 e fevereiro de 2008) e grupo II (287 gestantes, analisadas de março a outubro de 2008). No grupo I, foram encontrados 53,1% de gestantes IgG reagente e IgM não reagente, 1,9% de IgG e IgM reagentes, 0,3% de IgG não reagente e IgM reagente e 44,7% de IgG e IgM não reagentes. No grupo II foram 55,1% de IgG reagente e IgM não reagente e 44,9% de IgG e IgM não reagentes. As variáveis associadas à infecção foram: residência em área rural, gestantes entre 35-40 anos de idade, baixa escolaridade, baixa renda familiar, mais de uma gravidez, consumo de água não proveniente do sistema de abastecimento público de água e hábito de manipular terra ou areia. As orientações sobre medidas de prevenção primária e o acompanhamento sorológico trimestral das gestantes no grupo de risco são medidas importantes para prevenir a toxoplasmose congênita.

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