

## Risk factors for recent toxoplasma infection in pregnant women in Naples

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(Accepted 9 January 1996)

### SUMMARY

Effective primary prevention of congenital toxoplasmosis requires up to date information on locally relevant risk factors for infection in pregnant women. In Naples, risk factors for toxoplasma infection were compared in recently infected women (as assessed by detection of specific IgM in serum) and susceptible, IgG negative women. Recent infection was strongly associated with frequency of consumption of cured pork and raw meat. Eating cured pork or raw meat at least once a month increased the risk of toxoplasma infection threefold.

This simple study design for determining locally relevant sources of toxoplasma infection is the first report of cured pork as a risk factor for infection. Further research is required to determine cyst viability in cured pork products. Our findings suggest that in southern Italy, cured pork and raw meat should be avoided by susceptible pregnant women.

### INTRODUCTION

Prenatal screening for toxoplasma infection is widespread in Europe. A principal aim of screening is to identify susceptible pregnant women and offer advice on how to avoid infection. Toxoplasma infection can be acquired by ingestion of tissue cysts in undercooked meat or oocysts in cats' faeces or contaminated soil [1]. However, the marked variation in the prevalence of toxoplasma infection by region [1, 2], age and time [3], suggests that the relative importance of these sources of infection varies between populations. Strategies to prevent congenital toxoplasmosis based on advice on how to avoid primary toxoplasma infection are unlikely to be effective unless the locally relevant sources of infection are known and explained to susceptible women.

In this study of pregnant women in Naples, we used

toxoplasma specific IgM as a measure of recent infection and compared risk factors in recently infected and susceptible women.

### METHODS

All women admitted to the postnatal ward at the obstetric clinic of Federico II Medical School in Naples between November 1991 and June 1994 were invited to participate. A standard questionnaire including details of the women's diet, cat ownership and contact with soil with frequencies of exposure categorized as shown in Table 1, was administered at the bedside. A combined score was constructed to reflect the overall frequency of cured (dried and salted) pork or raw meat consumption (see Table 2).

Serum samples were analysed in duplicate for toxoplasma specific IgG and IgM antibodies using an immuno-capture assay manufactured by Sclavo [4, 5].

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Table 1. *Distribution of all pregnant women and IgG positive women studied in Naples (1991-4) according to risk factors*

| Risk factors                | All women<br>(n = 3518) |         | IgG positive<br>(n = 1422) |         | Percent of women<br>who were IgG positive<br>(total = 40.4%) |
|-----------------------------|-------------------------|---------|----------------------------|---------|--|
|                             | Number                  | Percent | Number                     | Percent |  |
| <b>Age (years)</b>          |                         |         |                            |         |  |
| < 20                        | 131                     | 3.7     | 41                         | 2.9     | 31.3   |
| 20-24                       | 813                     | 23.1    | 297                        | 20.9    | 36.5   |
| 25-29                       | 1334                    | 37.9    | 522                        | 36.7    | 39.1   |
| 30-34                       | 821                     | 23.3    | 351                        | 24.7    | 42.7   |
| ≥ 35                        | 419                     | 11.9    | 211                        | 14.8    | 50.4   |
| <b>Parity</b>               |                         |         |                            |         |  |
| 1st child                   | 1719                    | 48.9    | 502                        | 42.3    | 35.0   |
| 1 previous child            | 1139                    | 32.4    | 466                        | 32.8    | 40.9   |
| 2 or more                   | 660                     | 18.7    | 354                        | 24.9    | 53.6   |
| <b>Residence*</b>           |                         |         |                            |         |  |
| Urban                       | 2921                    | 83.0    | 1149                       | 80.8    | 39.3   |
| Rural                       | 597                     | 17.0    | 273                        | 19.2    | 45.7   |
| <b>Ever lived on a farm</b> |                         |         |                            |         |  |
| Never                       | 1896                    | 53.9    | 729                        | 51.3    | 38.4   |
| At least 1 week             | 996                     | 17.8    | 405                        | 28.4    | 46.0   |
| At least 1 month            | 626                     | 28.3    | 288                        | 20.3    | 40.7   |
| <b>Gardening</b>            |                         |         |                            |         |  |
| Never/rarely                | 2512                    | 71.4    | 984                        | 69.2    | 39.2   |
| Once a month                | 577                     | 16.4    | 241                        | 16.9    | 41.8   |
| Once a week                 | 429                     | 12.2    | 197                        | 13.9    | 46.0   |
| <b>Ever owned a cat</b>     |                         |         |                            |         |  |
| Never                       | 3182                    | 90.4    | 1281                       | 90.0    | 40.3   |
| Ever                        | 336                     | 9.6     | 141                        | 9.9     | 42.0   |
| <b>Eat raw meat</b>         |                         |         |                            |         |  |
| Never/rarely                | 2582                    | 73.4    | 1036                       | 72.9    | 40.1   |
| More than once a month      | 704                     | 20.0    | 287                        | 20.2    | 40.8   |
| More than once a week       | 232                     | 6.6     | 99                         | 7.0     | 42.7   |
| <b>Eat cured pork†</b>      |                         |         |                            |         |  |
| Never/rarely                | 2128                    | 60.5    | 779                        | 54.8    | 36.6   |
| More than once a month      | 970                     | 27.6    | 433                        | 30.4    | 44.6   |
| More than once a week       | 420                     | 12.0    | 210                        | 14.8    | 50.0   |

\* Area of residence defined according to the 1991 census: urban = < 10%, rural = 10% or more of the workforce employed in agriculture.

† Cured (dried and salted) pork meat of any type whether locally produced or bought in the supermarket (e.g. salami, coppa, sausages, salsiccia).

IgM and IgG positive women (IgG titre  $\geq 15$  IU/ml) were defined as recently infected, IgG negative (titre < 15 IU/ml) women as susceptible and IgG positive and IgM negative women as previously infected.

Univariate and multivariate logistic regression models were used to estimate odds ratios for recently infected (IgM and IgG positive) compared with susceptible women (IgG negative) using the GLIM statistical package [6]. Exposures were grouped into

'at least once a month' and 'less than once a month' categories. In multivariate models a step down approach excluded variables not significant at the 5% level.

## RESULTS

Of the 3778 women approached, 3765 (94%) agreed to be interviewed and tested for toxoplasma anti-

Table 2. Number and proportion of recently infected pregnant women and odds ratios for recent infection according to risk factors in Naples (1991-4)

| Risk factors           | Number recently infected,<br><i>n</i> = 42 IgM positive<br>susceptibles (%) | Odds ratio for recent<br>infection – IgM positivity<br>(95% confidence intervals) |
|------------------------|---|---|
| Age (years)            |   |   |
| < 20                   | 2 (2.17)  | ] 1.0<br>] 0.6 (0.9, 1.1)   |
| 20-24                  | 15 (2.82)   |   |
| 25-29                  | 19 (2.29)   |   |
| 30-34                  | 4 (0.84)  |   |
| ≥ 35*                  | 2 (0.95)  |   |
| Parity                 |   |   |
| 1st child              | 28 (2.45)   | ] 1.0<br>] 0.6 (0.3, 1.1)   |
| 1 previous child       | 7 (1.03)  |   |
| 2 or more              | 7 (2.24)  |   |
| Residence†             |   |   |
| Urban                  | 34 (1.88)   | 1.0   |
| Rural                  | 8 (2.41)  | 1.3 (0.6, 2.8)  |
| Ever lived on a farm   |   |   |
| Never                  | 21 (1.80)   | ] 1.0<br>] 1.3 (0.7, 2.3)   |
| At least 1 week        | 12 (3.43)   |   |
| At least 1 month       | 9 (1.50)  |   |
| Gardening              |   |   |
| Never/rarely           | 24 (1.55)   | ] 1.0<br>] 2.0 (1.1, 3.7)   |
| Once a month           | 11 (3.17)   |   |
| Once a week            | 7 (2.93)  |   |
| Ever owned a cat       |   |   |
| Never                  | 39 (2.01)   | 1.0   |
| Ever                   | 3 (1.52)  | 0.7 (0.2, 2.4)  |
| Eat raw meat           |   |   |
| Never/rarely           | 22 (1.40)   | ] 1.0<br>] 2.6 (1.4, 4.7)   |
| More than once a month | 9 (2.11)  |   |
| More than once a week  | 11 (7.64)‡  |   |
| Eat cured pork§        |   |   |
| Never/rarely           | 16 (1.17)   | ] 1.0<br>] 2.9 (1.6, 5.5)   |
| More than once a month | 14 (2.54)   |   |
| More than once a week  | 12 (5.41)‡  |   |

\* Denominator for susceptible women: number IgG negative (total 2906) + number IgM positive (total 42) in each exposure category.

† Area of residence defined according to the 1991 census: urban = < 10%, rural = 10% or more of the workforce employed in agriculture.

‡  $\chi^2$  for trend;  $P < 0.001$ .

§ Cured (dried and salted) pork meat of any type whether locally produced or bought in the supermarket (e.g. salami, coppa, parma ham, salsiccia).

bodies. Nearly half were primiparous. Complete results were available for 3518 women of whom 1422 were IgG positive, 42/3518 (1.2%) were recently infected (IgM + IgG positive), 1380 (39%) were previously infected (IgG positive, IgM negative) and 2096 (60%) were susceptible (IgG negative). Less than half (19/42 (45%)) of the recently infected women had been tested and treated during pregnancy.

The distribution of the study population and previously and recently infected women according to the risk factors measured is shown in Table 1.

On univariate analysis, IgM positivity was significantly associated with eating cured pork (odds ratio 2.9, 95% confidence interval: 1.6-5.5), eating raw meat (odds ratio 2.6, 95% CI: 1.4-4.7) and gardening (odds ratio 2.0, 95% CI: 1.1-3.7), but not cat

Table 3. Crude odds ratio for recent infection in pregnant women in Naples (1991–4) associated with reported frequency of eating cured pork and/or raw meat

| Score | Frequency of eating cured pork and/or raw meat                            | Number exposed (%) | Number IgM positive | Odds ratio | Confidence interval (95%) |
|-------|---|--------------------|---------------------|------------|---------------------------|
| 1     | Never/rarely  | 1167 (54.6)        | 12                  | 1.0        | —                         |
| 2     | Cured pork or raw meat monthly  | 462 (21.6)         | 11                  | 2.4        | 1.0–5.3                   |
| 3     | Cured pork or raw meat weekly, or pork and raw meat monthly               | 340 (15.9)         | 5                   | 1.4        | 0.5–4.1                   |
| 4     | Cured pork or raw meat weekly and raw meat or pork monthly (respectively) | 109 (5.1)          | 8                   | 7.6        | 3.1–19.1                  |
| 5     | Cured pork and raw meat weekly  | 60 (2.8)           | 6                   | 10.7       | 3.9–29.6                  |
|       | Total   | 2138* (100)        | 42                  |            |                           |

\* Total 2138: 2096 susceptible women (IgG negative) and 42 recently infected (IgM + IgG positive).

ownership (odds ratio 0.7, 95% CI: 0.2–2.4) (see Table 2). A significant linear trend was found between recent infection and the frequency of eating cured pork and raw meat.

Variables in Table 1 were included in a step down multivariate model but age group (< 25 years or ≥ 25 years), parity (primiparous or multiparous), urban residence, cat ownership, living on a farm and gardening were not significant ( $P > 0.05$ ). Eating cured pork at least once a month remained highly significant after adjustment for cat ownership, gardening and raw meat consumption ( $\chi^2 = 5.8$ , 1 D.F.;  $P = 0.02$ ). Eating raw meat was also a significant risk factor after adjustment for cat ownership and gardening, but not when cured pork was included in the model suggesting that cured pork and raw meat consumption were strongly correlated.

The combined score for meat consumption (Table 3) provided a model which was not significantly improved by inclusion of gardening or cat ownership ( $\chi^2 = 2.8$  and 0.6 respectively, 1 D.F.). The risk of recent infection associated with meat consumption was essentially explained by a linear effect with the odds ratio increasing by a factor of 1.77 (95% confidence limits: 1.4, 2.2) per unit increase in score (nonlinear component  $\chi^2 = 5.4$ , 3 D.F.;  $P = 0.14$ ). There was no statistical interaction between any variables for meat consumption, gardening and cat ownership.

Overall, 45% of susceptible women (971/2138) reported eating cured pork or raw meat at least once a month (odds ratio 3.1; 95% confidence limits: 1.6, 6.0). Based on these results and assuming a causal relationship, avoidance of monthly consumption of

cured pork and raw meat by pregnant women in Naples could reduce the risk of toxoplasma infection by approximately 48% (95% confidence limits: 32%, 84%) [7].

## DISCUSSION

Eating cured pork or raw meat were the major risk factors for recent toxoplasma infection in pregnant women in the Naples area. While it is uncertain whether these findings would be generalizable to other populations, the study demonstrates a simple method for identifying locally relevant risk factors.

Raw meat is a well established source of infection [1, 2] but this is the first report to identify eating cured pork as a risk factor for toxoplasma infection. Further studies are required to confirm this association and to demonstrate the viability of tissue cysts in cured pork products. Cysts are rendered non-infective after 12 h in 1.1% saline (1 g/100 g) at 18–22 °C but may survive prolonged salting and drying at lower temperatures [8]. A study of infected meat showed non-viability of tissue cysts after curing with more than 10% added salt and sucrose [9]. However, this and other experiments [10] do not reflect conditions in which some cured pork products are produced. In southern Italy, cured pork products usually contain 1% salt to fresh weight (in line with government requirements, personal communication: G. A. Polidori), are stored at less than 12 °C and may be eaten within 10 days of slaughter.

In general, infection is more likely from pork, lamb and goats' meat than beef, liver and heart muscle than skeletal muscle [2, 11, 12], and products constituted

from more than one animal. Indoor farming, prolonged meat freezing below  $-20^{\circ}\text{C}$ , irradiation and heating above  $65^{\circ}\text{C}$  reduce the risk of infection [2, 7, 9, 13].

If toxoplasmosis can be acquired from cured pork products, the risk of infection is likely to be closely associated with methods of farming and curing and with the amount consumed. However, pending further evidence of a causal relationship, our findings suggest that if susceptible pregnant women in Naples avoid eating cured pork or raw meat, the risk of primary infection could be halved.

The lack of evidence for an association with cat ownership has been confirmed by other studies [14] but further investigation of the risk of infection associated with contact with soil or cats' faeces is required.

Precise information on the mean duration and distribution of IgM persistence after primary infection is not available for any IgM tests [15]. In a UK study, all of 10 sera taken 6 months after onset of toxoplasma lymphadenopathy were negative using an in-house ELISA [16] but such findings are likely to vary between tests. In our study, misclassification of IgM positive women with prolonged duration of IgM [17] as recently infected would have reduced the differences in exposures between the comparison groups. Recall bias is likely to have had minimal effect as less than half the IgM positive women knew their result, all were asymptomatic and delivered asymptomatic babies.

Use of IgM and IgG to compare exposures in recently infected and uninfected pregnant women is a simple approach for identifying locally relevant risk factors to inform primary prevention strategies. In southern Italy, pregnant women should be advised to avoid eating cured pork or raw meat.

#### ACKNOWLEDGEMENTS

The study was partly supported by the European Network on Congenital Toxoplasmosis, a Concerted Action under the European Union BIOMED1 programme (contract number BMH1-CT92-1572). Ruth Gilbert was funded by a Wellcome Trust training fellowship in clinical epidemiology. We are grateful to Professor Martinelli for access to patients and Dr Marisa Del Pezzo for microbiological testing.

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