Study on abortion associated with *Toxoplasma gondii* in women based on PCR detection of aborted placenta and maternal serology in Ardabil, Iran

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What is Toxoplasmosis?

- It is a zoonotic infection caused by the parasite *Toxoplasma gondii*

The protozoan *Toxoplasma gondii* is a

- ✓ coccidian
- ✓ obligate
- ✓ intracellular parasite

- Toxoplasmosis can be categorized into four groups:
  - ✓1- Acquired in the immunocompetent patient
  - ✓2 – Acquired or reactivated in the immunodeficient patient
  - ✓3 – Congenital
  - ✓4 – Ocular

Congenital toxoplasmosis

generally occurs when a woman is newly infected with *T. gondii* during pregnancy and can lead to a wide variety of manifestations in the fetus and infant including spontaneous abortion, still-birth or a live infant with classic signs of congenital toxoplasmosis.

Congenital toxoplasmosis is a problem in 1-5/1000 pregnancies

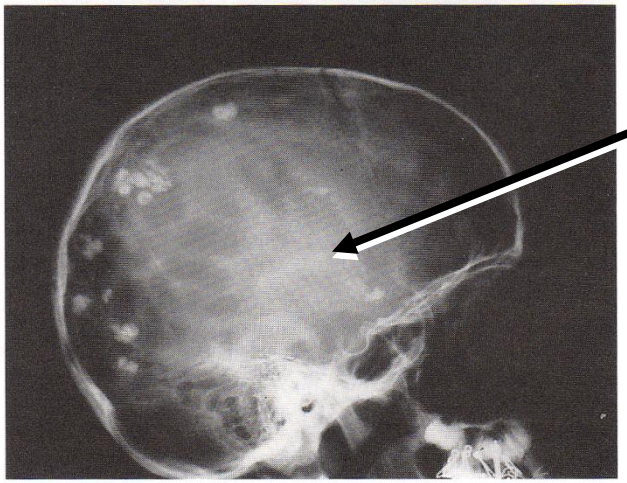


Fig. 16.10 Intracerebral calcification discovered fortuitously in a 10 year old girl, on a dental panoramic radiograph asked for by a dentist. The girl had unilateral retinochoroiditis and an IQ of 80. (Courtesy of Dr J. Couvreur).

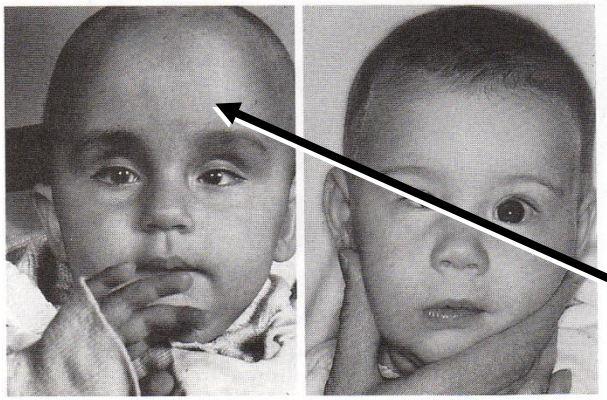


Fig. 16.9 Congenital toxoplasmosis in children. Hydrocephalus with bulging forehead (left) and microophthalmia of the left eye (right). (Courtesy of Dr J. Couvreur).

* **<u>Intracerebral calcification</u>.**

- Hydrocephaly
- Microcephaly
- Chorioretinitis
- Intracerebral calcification

- One third of mothers who acquire a primary *T.gondii* infection during pregnancy transmit the infection to their fetuses .
- In *Toxoplasma* - induced abortion, a combination of diagnostic techniques is necessary for accurate diagnosis.

Diagnosis:

- Serological tests
- Polymerase chain reaction (PCR)
- Histological demonstration of the parasite or its antigens
- Isolation of the organism

Methods of diagnosis and their interpretations may differ for each clinical category.

MATERIALS AND METHODS

This study has been performed on two hundred women in different gestational age who experienced abortion and referred to Gynecological department of Alavi hospital in Ardabil Iran.

Blood samples were tested for specific anti-*Toxoplasma* antibodies by ELISA and the placenta was tested by nested PCR.

sample collection

- For each patient, a questionnaire including the mother's age, gestational age and the history of prior abortion and disease was completed.

sample collection

- 3 cc of venous blood was drawn from each patients and their serums were isolated. About 20 g of the placenta sample of the same patients were cut in sterilized conditions and stored together with the serum samples in the temperature of -20 °C until conducting the tests.

sample collection

- The sera of all cases were tested for the presence of specific IgM and IgG anti-*Toxoplasma* antibodies via ELISA kits (Biokit, Spain) according to the manufacturer's instructions.

DNA extraction and PCR detection:

T. gondii DNA was extracted from placenta of women who had experienced abortion using the QIAamp DNA mini kit (Qiagen, France).

Detection procedures sets were used for amplifying fragments of 529bp element, described by su et al (2009)

Results

In this study

- women age range was from 16 to 41 years with a mean of 30 years.
- 53% were in their first trimester of pregnancy
- 73 % has one or no child
- 71% had no experience of miscarriage
- 84% had history of no diseases except miscarriage.

Serology

53% of serum samples were positive for anti-*Toxoplasma* antibodies

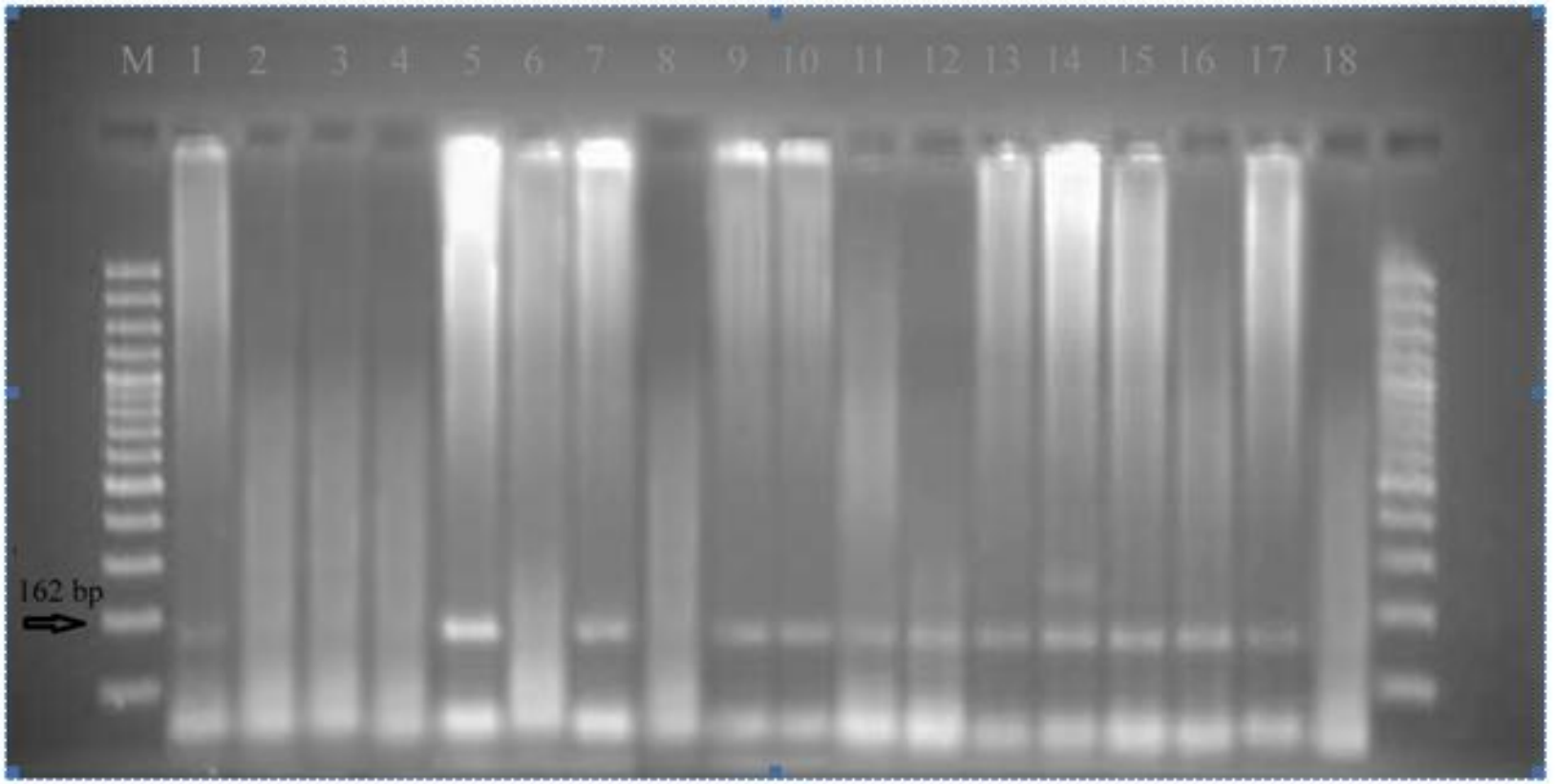
- 43% were positive for only anti-*Toxoplasma* IgG antibodies
- 6% were positive for both anti-*Toxoplasma* IgG and IgM antibodies.
- 4% were positive for only anti-*Toxoplasma* IgM antibodies.

Both samples either with anti-*Toxoplasma* IgM antibodies and both anti-*Toxoplasma* IgG and IgM antibodies were recorded as positive (10%).

PCR

- In PCR, the positivity and negativity was based on detection of special visible bands (162 bp for the first run and 450 bp for the second run).
- Both samples– with the 450 bp visible band and without– were tested for the second run of PCR to detect the 162 bp band. Both samples either with two bands visible and or just one 162 bp band visible were recorded as positive. Negative samples displayed neither of the two special bands.

- *Toxoplasma* infection was diagnosed in 21 out of 200 (10·5%) aborted placenta samples based on PCR (Figure 1). In 16 of the positive samples both the 162 bp and the 450 bp bands were detectable, but in 5 positive specimens, just the 162 bp band was detected in Nested-PCR. Four maternal sera were positive in ELISA but not in the PCR attempted on the reported aborted placenta



Agarose gel electrophoresis of nested PCR products of tissue samples of aborted placenta with the internal 529bp element primers. Lane M molecular marker, lane 1 *T. gondii* positive control (type 1; RH strain) ,lane 18 negative control, lane 2-17 samples

- Among 21(10.5%) women who recorded positive results in Nested-PCR, it was found that
- 5(23.8%) of them appeared with IgM+
- 1 (4.76%) with IgG+
- 4 (19.04%) with no anti-*Toxoplasma* antibodies.

- In addition 11(52.38%) of a positive Nested-PCR results revealed both (IgM+ and IgG+) antibodies.

Prevalence and agreement

- Maternal seroprevalence was 5·8% to 14·2% (95% confidence interval) and the estimated abortion prevalence associated with *T. gondii*, based on PCR was calculated as being from 6.3% to 14.7% with 95% confidence interval. Results show moderate logical agreement between the 2 different tests ($\kappa = 0 \cdot 44$).

		No	PCR		ELISA	
			Positive	Negative	Positive	Negative
Age groups(yr)	≤20	43	4	37	5	52
	20-30	88	10	76	9	62
	>30	69	7	61	6	66
Gestational age	1st trimester	106	11	95	9	97
	2th trimester	62	8	54	10	42
	3th trimester	32	2	30	1	41
History of abortion	Yes	58	3	55	2	56
	No	142	18	124	18	124

According to the result of this study, about 10·5 %(6·3%–14·7% with a 95% confidence interval) of abortions were associated with this organism, although the involvement of other pathogens could not be ruled-out.

In our study it was estimated at moderate agreement between the 2 different tests with 95% confidence interval ($\kappa = 0 \cdot 44$).

These findings increase the necessity for further work to clarify the precise mechanisms of route of transmission and its occurrence from generation to generation following primary infection.

Although serological testing has been one of the major diagnostic for toxoplasmosis, it has many limitations:

It may fail to detect specific anti-*Toxoplasma* IgM or IgG during the active phase of infection, because these antibodies may not be produced until after several weeks of parasitemia.

Furthermore, the test may fail to detect *T. gondii* infection in certain immune compromised patients due to the fact that the titers of specific anti-*Toxoplasma* antibodies may fail to rise in this type of patient.

Detection of *T.gondii* DNA using nPCR minimizes the problems faced when using sero diagnostic assays and facilitates diagnosis in difficult cases. Therefore, the negative results obtained by both PCR and ELISA rule out an infection in women who had abortion.

Toxoplasma DNA was detected in 4.26% seronegative women; it could correspond to a very recent infection at the time of serological leading to an insufficient production of immunoglobulin not detected by serology, or other explanation that those patients are not able to produce specific antibodies, representing a state of immunodeficiency.

- A positive serological result is only indicative of infection, whereas direct detection of *T. gondii* in clinical samples confirms the parasite presence leading to the diagnosis of primary, reactivated or chronic toxoplasmosis .

- In the present research there was no significant association between history of abortion and existence of *Toxoplasma*.

- Our findings show that toxoplasmosis acts as a warning and main risk factor for increase the possibility of abortion in pregnant woman in this region of Iran, therefore we recommend for each abortion, to improve detection sensitivity, molecular examination of placenta be done even if prenatal serological test was negative.

Thank you for your attention