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# Association of Maternal *Toxoplasma Gondii* Molecular and Serological Positivity With Child's Gross-Motor Development and Behavior in Tribal Regions of Gujarat, India: A Prospective Study

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## 1 | Introduction

*Toxoplasma gondii* is a protozoan parasite causing toxoplasmosis in humans, with lifelong presence in brain and muscular tissues [1, 2]. Disease is mostly asymptomatic with infection largely correlating with changes in human personality [3, 4]. *T. gondii* infection can be acquired from environment and during pregnancy when the parasite can be transmitted to the fetus through the placenta resulting in congenital toxoplasmosis. National and international studies including our previous community study have assessed *Toxoplasma* prevalence in pregnant women through molecular and serological methods [5–8]. Follow-up studies have explored severe or more apparent outcomes among children born to *Toxoplasma*-positive mothers, such as hearing-vision deficiencies including chorioretinitis [9, 10]. Longer pregnancy, slower fetal development, and slower post-natal gross-motor development have been associated with children born to *Toxoplasma*-positive mothers [11, 12]. However, similar profiling of development characteristics in a developing country like India is lacking. In addition, temperament characteristics among children born to *Toxoplasma*-positive mothers are unclear.

Therefore, the objective of this small-scale, exploratory study was to explore the association of maternal *Toxoplasma* positivity with child's gross-motor development and temperament through structured, well-established, parent-reported questionnaires. This is a nested investigation involving mothers from a previously completed prospective pregnancy cohort study [6].

## 2 | Methods

### 2.1 | Study Area and Subject Recruitment

*T. gondii* prevalence study was previously conducted among pregnant women in Modasa-Bhiloda blocks, Aravalli district, Gujarat, India covering > 100 villages through universal sampling [6, 13]. A combination of nested PCR assays detecting B1 gene and ELISA assays detecting *Toxoplasma*-specific IgM and IgG antibodies were employed. The study revealed high seroprevalence (76.5%) owing to poor personal and environmental hygiene in these tribal regions [6, 13] providing relevance for conducting the present follow-up study. Following categories of *Toxoplasma* positivity in mothers were observed: PCR−IgG+ (40.9%), PCR+IgG+ (34.5%), PCR+IgG− (3.1%), and PCR+IgG +IgM+ (1.1%). Based on this data set of mothers, the target population for the present study included only the children born to the two highest prevalence categories of *Toxoplasma* positivity (PCR+IgG+ and PCR−IgG+). Villages demonstrating the highest prevalence in both categories of *Toxoplasma* positivity were included in the present study. For optimal use of

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resources, *Toxoplasma*-positive mothers in the surrounding villages were also included due to geographical proximity for easy movement of data collectors between villages. Within these selected villages, all women who participated in the previous prevalence study were contacted, demonstrating the three categories of *Toxoplasma* status (PCR+IgG+, PCR−IgG+, PCR−IgG−) without adverse birth outcomes. However, not all women in each village were available due to migration. Children born to available *Toxoplasma*-positive (PCR+IgG+ [$n = 95$] and PCR−IgG+ [$n = 94$]) and *Toxoplasma*-negative (PCR−IgG− [$n = 59$]) mothers were enrolled. The sample size constituted 30% of the pregnancy cohort study population demonstrating these categories of *Toxoplasma* status without adverse birth outcomes. Enrolled children included infants and toddlers between the ages of 6 and 28 months old.

## 2.2 | Measures

Based on the prevalent modes of environmental transmission mechanisms, a questionnaire examining household interaction with cats, milk–water source, and food consumption was administered to parents. Children's bio-parameters including age, height, weight, and gross-developmental milestones were noted. Children's height and weight were measured by the data collector with the help of a measuring tape and weighing scale, respectively. Infant behavior questionnaire- revised, very short version (IBQ-R-VS) was administered to parents, measuring infant's ($\leq 12$ months old) three scales of temperament; surgency, effortful control, and negative affect. The questionnaire and scoring guidelines were obtained from Dr. Sam Putnam, Bowdoin College, who originally developed the questionnaire [14]. The questionnaire was translated into the local language (Gujarati), back-translated, and pretested in a pilot study. Cronbach's alpha coefficient was determined for scales; surgency, negative affect, and effortful control as 0.56, 0.66, and 0.55, respectively. All questionnaires were administered to parents and responses were recorded by a trained data collector. Two-sided significance was determined for all statistical tests and $p \leq 0.05$ was considered as statistically significant. Shapiro–Wilk test of normality was performed to determine the nature of distribution among the groups being compared. All statistical analyses were performed using the Statistical Package of Social Science (IBM SPSS Statistics 26).

## 2.3 | Ethical Considerations

The study was approved by the Institutional Ethics Committee of the Indian Institute of Public Health Gandhinagar (approval code: 15/2020-21). The study implications were explained to the parents of study participants in the local language (Gujarati). Written informed consent was obtained from parents of study participants in Gujarati. For illiterate parents, a thumbprint was taken along with the witness's signature. No invasive procedures or interventions were performed.

## 3 | Results

There were no significant differences in the prevalence of any of the household-associated infection risk factors among the categories of children born to *Toxoplasma*-positive mothers (IgG+, PCR+IgG+, PCR−IgG+) and *Toxoplasma*-negative mothers (PCR−IgG−) (Supporting Information: Table S1). Comparing children born to *Toxoplasma*-positive and *Toxoplasma*-negative groups, there were no significant differences in bio-parameters such as age, low birth weight status, weight, and height (Table 1). Adjustment for confounders such as gender, low birth weight status, and age did not result in any significant association of mothers' *Toxoplasma* positivity with the child's weight or height.

In contrast to another study [12], gross motor skill development did not show significant differences between categories of children born to *Toxoplasma*-positive and *Toxoplasma*-negative mothers (Table 1). Ages of achieving indicated milestones are within the age cut-offs for normal development as per the Indian Academy of Pediatrics Guidelines [15], except for the median age of holding head which is delayed by a month from the cut-off in all the groups. None of the children had vision and hearing deficiencies.

IBQ-R-VS responses collected from infants ($\leq 12$ months old) revealed significantly higher levels of negative affect scores in infants born to PCR+IgG+ mothers compared to both infants born to PCR−IgG− mothers, $p = 0.01$ and infants born to PCR−IgG+ mothers, $p = 0.02$ (Table 2). No significant changes were observed in other temperament scales.

## 4 | Discussion

Our study has identified similar exposure to infection risk factors between the households of *Toxoplasma*-positive (PCR+IgG+, PCR−IgG+, and IgG+) and *Toxoplasma*-negative mothers (PCR−IgG−). Given this scenario, our study demonstrates that the higher negative affect scores are associated with infants born to PCR+IgG+ mothers, not with infants born to PCR−IgG+ mothers. Although the biological significance of these subsets is presently unknown, our study indicates the importance of performing both PCR and serological assays for better understanding of the relationship of maternal *Toxoplasma* positivity during pregnancy with a child's temperament characteristics. Large-scale studies are needed to confirm these findings. In addition, longitudinal studies are needed to assess the temperament of these children as they enter adolescence and adulthood.

High negative affectivity has been identified in children with attention-deficit/hyperactivity disorder (ADHD), a developmental and behavioral disorder [16, 17]. Interestingly, increased levels of severe form of ADHD were correlated with *Toxoplasma* seropositivity in children [18, 19]. Although we did not clinically evaluate ADHD in this study, future studies will need to explore the predisposition to development of ADHD in children demonstrating both high negative affectivity and *Toxoplasma* seropositivity to understand the role of *Toxoplasma* in regulating behavioral functions. Additionally, biological and environmental factors contribute to negative affectivity, including parenting behaviors, household chaos, and maternal emotion expressivity [20], which were not evaluated in the present study.

There was a lack of significant difference in children's bio-parameters between the groups. Although large-scale



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**TABLE 1** | Study participant bio-parameters.

| Child's bio-parameters | Mother's *Toxoplasma* infection status | | | | | |
|---|---|---|---|---|---|---|
| | IgG+ | PCR+IgG+ | PCR−IgG+ | PCR−IgG− | *p*-value[a] | *p*-value[b] |
| | Frequency (% within mother's *Toxoplasma* infection status category) | | | | | |
| Born as low birth weight infant | ***n* = 189** | ***n* = 95** | ***n* = 94** | ***n* = 59** | | |
| | 39 (20.6) | 18 (18.9) | 21 (22.3) | 15 (25.4) | 0.44 | 0.63 |
| | Median (interquartile range), *n* | | | | *p*-value[c] | *p*-value[d] |
| Age (months) | 16 (6), 189 | 16 (6), 95 | 17 (8), 94 | 16 (6), 59 | 0.69 | 0.89 |
| Height (cm) | 76 (9), 180 | 76 (8), 93 | 76 (9), 87 | 73 (9), 54 | 0.19 | 0.4 |
| Weight (kg) | 10 (2), 181 | 10 (1), 93 | 10 (2), 88 | 10 (1), 54 | 0.65 | 0.67 |
| Age during which infant learned to lift his/her head independently (months) | 5 (1), 189 | 5 (1), 95 | 5 (1), 94 | 5 (1), 59 | 0.85 | 0.66 |
| Age during which infant learned to move/turn around independently (months) | 6 (0), 189 | 6 (0), 95 | 6 (0), 94 | 6 (0), 59 | 0.49 | 0.79 |
| Age during which infant learned to sit independently (months) | 9 (1), 188 | 9 (1), 94 | 9 (1), 94 | 9 (1), 59 | 0.62 | 0.84 |
| Age during which infant learned to kneel independently (months) | 8 (1), 187 | 8 (1), 94 | 8 (1), 93 | 8 (1), 59 | 0.51 | 0.49 |
| Age during which infant learned to walk independently (months) | 12 (1), 176 | 12 (1), 94 | 12.5 (1), 82 | 12 (1), 57 | 0.92 | 0.99 |

*Note:* $p > 0.05$, Not Significant.
[a]Pearson chi-square test comparing IgG+ and PCR−IgG− groups.
[b]Pearson chi-square test comparing PCR+IgG+, PCR−IgG+, PCR−IgG− groups.
[c]Mann–Whitney test comparing IgG+ and PCR−IgG− groups.
[d]Kruskal–Wallis test comparing PCR+IgG+, PCR−IgG+, PCR−IgG− groups.

**TABLE 2** | Comparison of temperament scores among infants born to *Toxoplasma*-positive and *Toxoplasma*-negative mothers.

| Temperament scales | Maternal *Toxoplasma* infection status | | | |
|---|---|---|---|---|
| | IgG+ (*n* = 22) | PCR+IgG+ (*n* = 7) | PCR−IgG+ (*n* = 15) | PCR−IgG− (*n* = 5) |
| | Infant temperament scale scores | | | |
| | | | Mean (SEM)[a,b] | |
| Surgency | 4.78 (0.08) | 4.77 (0.1) | 4.79 (0.11) | 4.88 (0.24) |
| Effortful control | 4.61 (0.07) | 4.57 (0.12) | 4.63 (0.09) | 4.57 (0.24) |
| Negative effect | **Mean (SEM)**[b] | | **Median (Interquartile range)**[c] | |
| | | **4.67 (0.33)**[d,e] | 4.33 (0.42) | 3.92 (0.63) |
| | | | **Mean (SEM)** | |
| | 4.52 (0.1) | NA | NA | 4.07 (0.18) |

Abbreviation: NA = not applicable.
[a]ANNOVA test was performed for comparison between PCR+IgG+, PCR−IgG+, and PCR−IgG− groups.
[b]Independent *t*-test (equal variances) was performed for comparison between IgG+ and PCR−IgG− groups. Mean(standard error of mean) is shown.
[c]Kruskal–Wallis test, followed by Dunn–Bonferroni post-hoc test was performed for comparison between PCR +IgG+, PCR−IgG+, and PCR−IgG− groups.
[d]Comparison between PCR+IgG+ and PCR−IgG− groups demonstrating $p = 0.01$.
[e]Comparison between PCR+IgG+ and PCR−IgG+ groups demonstrating $p = 0.02$. Median (interquartile range) is shown. $p \leq 0.05$ was considered statistically significant.



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studies are needed to confirm, inherent similarities including food habits, malnutrition, and anemia [21] need to be considered.

In contrast to a Western study [12], our analysis did not show a significant difference in gross-motor skills such as the age at which the child learned to lift the head and move/turn around independently. It is possible that there are genetic and environmental factors that need to be considered [22]. A limitation of the study is that developmental features were not assessed comprehensively, for example, through questionnaires like "Ages and Stages" (ASQ), and Developmental Assessment Scale for Indian Infants (DASII) which have been established to detect developmental delay in Indian children [23].

There are other limitations in the study that children's *Toxoplasma* status could not be determined due to resource and logistical constraints, and could not be subsequently correlated with their temperament and corresponding mother's *Toxoplasma* status. Analyzing these aspects in future studies would provide more insight into the pathophysiological link between a mother's *Toxoplasma* status during pregnancy and subsequent child's behavior. Potential confounding factors such as parent's educational levels, family's emotional environment, and children's health factors were not evaluated. Another limitation is that the Cronbach's alpha values in the IBQ-R-VS questionnaire were not excellent in our study and future studies need to assess how the scale reliability in Gujarati-translated questionnaires could be further improved. It is possible that there are other cross-cultural nuances that need to be considered.

One of the strengths of this study is that this is a follow-up of a previously conducted large prospective study that assessed the prevalence of TORCH positivity among pregnant women. In the present study, as their children were being evaluated, mothers were enthusiastic in participating and forthcoming in their responses. Furthermore, the participating mothers were not aware of their *Toxoplasma* status, and therefore no inherent bias was involved while answering the questionnaires. Questionnaires were administered through a data collector belonging to the same study area who ensured that the parents understood the questions. In addition, infection risk factors were not significantly different between the *Toxoplasma*-positive and -negative mothers, allowing independent interpretation of the child's development and behavioral characteristics.

## 5 | Conclusions

The study indicates the importance of combinatorial assessment of molecular and serological testing for understanding the implications of *Toxoplasma* positivity during pregnancy through association with the infant's temperament characteristics. Large-scale studies are needed to determine if the PCR+IgG+ category of maternal *Toxoplasma* status poses an early increased risk for children to develop altered behavior later in life. Such a questionnaire-based approach involving the parents could directly impact understanding of *Toxoplasma* infection among the tribal community where currently, there is a lack of awareness about the disease.

### Author Contributions

**Aarthi Sundararajan:** conceptualization, methodology, funding acquisition, project administration, writing–original draft, writing–review and editing, supervision, data curation. **Kranti Vora:** conceptualization, supervision, project administration, writing–review and editing, methodology. **Shahin Saiyed:** methodology, project administration, writing–review and editing, data curation. **Senthilkumar Natesan:** conceptualization, writing–review and editing, supervision. **Vidhi Vaidya:** writing–review and editing, methodology.

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### Disclosure

The corresponding authors Aarthi Sundararajan and Kranti Vora affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

### Ethics Statement

The study was approved by the Institutional Ethics Committee of the Indian Institute of Public Health Gandhinagar (approval code: 15/2020-21).

### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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## Supporting Information

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