Prevalence of Toxoplasma gondii Infection in Brain and Heart by Immunohistochemistry in a Hospital-Based Autopsy Series in Durango, Mexico

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The presence of tissue cysts of Toxoplasma gondii has only poorly been investigated in autopsy series. We determined the presence of T. gondii cysts in a series of 51 autopsies in a public hospital using immunohistochemistry of brain and heart tissues. The association of tissue cysts with the general characteristics of the autopsy cases was also investigated.

Of the 51 cases studied, five (9.8%) were positive by immunohistochemistry for T. gondii cysts in the brain. None of the heart specimens was positive for T. gondii cysts. The presence of T. gondii cysts in brains did not vary with age, sex, birthplace, residence, education, occupation, or the presence of pathology in the brain. In contrast, multivariate analysis showed that the presence of T. gondii cysts was associated with undernourishment (OR = 33.90; 95% CI: 2.82–406.32; P = 0.005).

We demonstrated cerebral T. gondii cysts in an autopsy series in Durango City, Mexico. Results suggest that T. gondii can be more readily found in brain than in heart of infected individuals. This is the first report of an association between the presence of T. gondii in brains and undernourishment.

Keywords: Toxoplasma gondii, prevalence, postmortem examinations, heart, brain, immunohistochemistry

Introduction

Toxoplasma gondii (T. gondii) is a ubiquitous intracellular protozoan parasite [1]. About one-third of the population is infected with T. gondii [2], typically via ingestion of food or water contaminated with oocysts shed by T. gondii-infected cats [3] or eating undercooked or raw meat containing tissue cysts from T. gondii-infected animals [4, 5]. Following dissemination throughout the body, T. gondii forms cysts in muscular and central nervous system tissues resulting in latent infection [6]. The latent stage of infection is controlled by the immune system of the host [7]. Although most acute infections with T. gondii are asymptomatic, latently infected individuals, i.e., immunocompromised individuals, may develop reactivated disease manifesting in the eye or brain [3, 5, 8]. Infections with T. gondii have been associated with a number of mental disorders including memory impairment in seniors [9], schizophrenia [10], and effects on the rate of suicide attempts [11, 12], as well as traffic [10] and work [13] accidents. In addition, T. gondii infection may lead to heart disease including myocarditis [14, 15], pericarditis [16, 17], and acute heart failure [18].

The demonstration of T. gondii in brain and heart specimens in humans is typically limited to postmortem examinations. While T. gondii cysts can be recognized in routine stainings, T. gondii can best be demonstrated in tissues using immunohistochemistry [19]. This method has been successfully used for detection of T. gondii in AIDS autopsy series [20, 21]. However, to the best of our
knowledge, the use of this valuable method for the detection of *T. gondii* has not been reported in hospital-based autopsy series of routine postmortem examinations. The presence of *T. gondii* in brain tissue and heart muscle has not been investigated in detail thus far in Mexico. Therefore, we determined the presence of *T. gondii* in brains and heart muscle in an autopsy series in a public hospital in northern Mexico using immunohistochemistry. In addition, the association of the presence of *T. gondii* with general characteristics of the autopsy cases was investigated.

Materials and methods

**Autopsy series**

We studied a 6-year (2009–2014) hospital-based autopsy series in a public hospital (General Hospital of the Secretary of Health) in Durango City, Mexico. Inclusion criteria for the study cases were: 1) autopsy cases in the Pathology Department of the General Hospital, 2) with brain and heart tissues available, 3) any sex, and 4) any age. During the study period, 94 autopsies were performed. Of these 94 autopsies, only 51 had brain and heart tissues available. In total, 51 cases of postmortem examinations were included. Twenty-seven cases were females and 24 were males. Autopsy cases had an age from 3 days to 78 (mean 35.37 ± 24.62) years.

**Immunohistochemistry**

Archival formalin-fixed, paraffin-embedded sections of brain and heart from routine autopsy cases were included in the study. All autopsies were performed to clarify the diagnosis. Brain tissues were obtained from the left frontal lobe except one case (No. 21, choroid plexus). Heart tissues were obtained from the left auricular ventricular region. One brain tissue section and one heart tissue section of each of the 51 autopsy cases were analyzed with the aid of the Tinto Detector Immuno DNA System equipment (Bio SB, Santa Barbara, CA, USA) and Digital Pressure Cooker, Model PC-2000 (Bio SB). Immunohistochemistry was performed with the Mouse/Rabbit Immunodetector HRP/DAB (Bio SB). Immunostaining was performed on paraffin-embedded 2 μm tissue sections. The primary antibody “Toxoplasma gondii, rabbit polyclonal” (Bio SB) and the positive control “Toxoplasma gondii positive control slides” (Bio SB) were used. All assays were performed following the instructions of the manufacturer. A pathologist (LFSA) read the slides.

**Statistical analysis**

We performed the statistical analysis with the aid of the software: Epi Info version 7 and SPSS version 15.0. Bivariate analysis followed by multivariate analysis were used to examine the association of *T. gondii* infection and the characteristics of the autopsy cases. We used the two-tailed Fisher exact test for comparison of the frequencies among groups. As a strategy to select variables for the multivariate analysis, we included only variables that had *P* value ≤0.10 in the bivariate analysis. Odds ratio (OR) and 95% confidence interval (CI) were calculated by logistic regression analysis with the Enter method. We used the Hosmer–Lemeshow test to assess the goodness of fit of our regression model. Statistical significance was set at a *P* value of <0.05.

**Ethical considerations**

This study was approved by the Institutional Ethical Committee of the General Hospital of the Secretary of Health in Durango City, Mexico.

**Results**

Of the 51 autopsy cases studied, five (9.8%) were positive for *T. gondii* in the brain. None of the heart specimens was positive for *T. gondii*. A summary of the clinical and postmortem diagnoses of the 51 cases and their correlation with immunohistochemistry results is shown in Table 1.

| No. | Sex | Age | Diagnosis          | Brain histology | Presence of T. gondii |
|-----|-----|-----|--------------------|----------------|----------------------|
| 1   | F   | 76  | Rheumatoid arthritis | Cerebral ischemia | No                   |
| 2   | F   | 9   | Pulmonary embolism  | Respirator brain  | No                   |
| 3   | M   | 44  | Influenza           | Cerebral ischemia | No                   |
| 4   | M   | 38  | Influenza           | Cerebral ischemia | No                   |
| 5   | F   | 67  | Cervical cancer     | Cerebral ischemia | No                   |
| 6   | F   | 67  | Evans syndrome      | Normal           | No                   |
| 7   | M   | 0.4 | Battered child syndrome | Hemorrhage     | No                   |
| 8   | F   | 35  | Intestinal ischemia | Cerebral ischemia | No                   |

Table 1. Correlation of clinical characteristics and postmortem diagnoses with the presence of *T. gondii* in brain as detected by immunohistochemistry in an autopsy series.
Table 1. (cont.)

| No. | Sex | Age | Diagnosis                  | Brain histology                  | Presence of T. gondii |
|-----|-----|-----|----------------------------|----------------------------------|-----------------------|
| 9   | M   | 74  | Fibrinopurulent meningitis | Fibrinopurulent meningitis       | No                    |
| 10  | M   | 0.1 | Respiratory distress syndrome | Cerebral immaturity            | No                    |
| 11  | F   | 1.8 | Hepatitis, liver fibrosis   | Normal                           | No                    |
| 12  | F   | 38  | Pulmonary embolism          | Cerebral ischemia                | No                    |
| 13  | F   | 0.5 | Choledochal cyst            | Normal                           | No                    |
| 14  | F   | 34  | Sepsis                      | Cerebral ischemia                | No                    |
| 15  | M   | 17  | Bronchopneumonia            | Cerebral ischemia                | No                    |
| 16  | M   | 17  | Pulmonary embolism          | Normal                           | No                    |
| 17  | M   | 39  | Scorpion envenomation        | Normal                           | No                    |
| 18  | F   | 63  | Morbid obesity              | Normal                           | No                    |
| 19  | M   | 0.6 | Meningoencephalitis         | Viral meningoencephalitis        | No                    |
| 20  | M   | 0.2 | Intestinal perforation      | Normal                           | No                    |
| 21  | F   | 7   | Undernourishment            | Cerebral ischemia                | Yes                   |
| 22  | F   | 67  | Diabetes mellitus           | Normal                           | No                    |
| 23  | M   | 0.2 | Thrombocytopenia            | Normal                           | No                    |
| 24  | M   | 31  | Nephrotic syndrome          | Acute meningitis                 | No                    |
| 25  | F   | 22  | Bronchopneumonia            | Cerebral ischemia                | Yes                   |
| 26  | F   | 0.3 | Prematurity                 | Cerebral ischemia                | No                    |
| 27  | F   | 38  | Pneumonia                   | Cerebral ischemia                | No                    |
| 28  | M   | 68  | Polycythemia vera           | Cerebral edema                   | No                    |
| 29  | F   | 6   | Pulmonary hemorrhage        | Cerebral ischemia                | No                    |
| 30  | M   | 66  | Diabetes mellitus           | Cerebral thrombosis              | No                    |
| 31  | M   | 57  | Arterial hypertension       | Normal                           | No                    |
| 32  | M   | 78  | Arterial hypertension       | Cerebral ischemia                | No                    |
| 33  | M   | 45  | Diabetes mellitus           | Normal                           | No                    |
| 34  | M   | 51  | Adrenal carcinoma           | Cerebral edema                   | No                    |
| 35  | M   | 26  | Miliary tuberculosis        | Tuberculosis meningitis          | Yes                   |
| 36  | F   | 32  | Miscarriage                 | Normal                           | No                    |
| 37  | F   | 60  | Miliary tuberculosis        | Tuberculosis meningitis          | No                    |
| 38  | F   | 61  | Miliary tuberculosis        | Tuberculosis meningitis          | No                    |
| 39  | M   | 66  | Fibromuscular dysplasia     | Cerebral ischemia and thrombosis | No                    |
| 40  | F   | 59  | Systemic lupus erythematous | Normal                           | Yes                   |
| 41  | F   | 40  | Premature rupture of membranes | Normal                         | No                    |
| 42  | F   | 29  | Meningoencephalitis         | Acute and chronic meningitis     | No                    |
| 43  | M   | 44  | Fulminant varicella         | Cerebral ischemia                | No                    |
| 44  | F   | 38  | Miliary tuberculosis        | Cerebral ischemia and edema      | No                    |
| 45  | F   | 25  | HELLP syndrome              | Cerebral ischemia                | No                    |
| 46  | M   | 70  | Dermatomyositis             | Cerebral ischemia                | No                    |
| 47  | F   | 22  | Diabetes mellitus           | Cerebral ischemia                | No                    |
| 48  | M   | 13  | Thrombocytopenia            | Normal                           | No                    |
| 49  | F   | 23  | Eclampsia                   | Cerebral ischemia and edema      | No                    |
| 50  | M   | 16  | Bronchopneumonia            | Normal                           | Yes                   |
| 51  | F   | 22  | Pyogenic hepatic abscesses  | Cerebral ischemia                | No                    |
Figures 1 and 2 show *T. gondii* in choroid plexus (case No. 21) and in frontal lobe (case No. 50), respectively. *T. gondii* was found in astrocytes and neurons. None of the cases had clinical or postmortem diagnosis of toxoplasmosis. Table 2 shows the correlation of the general characteristics of the autopsy cases and *T. gondii* in brain. Presence of *T. gondii* in brains did not vary with age, sex, birthplace, residence, education, occupation, or the presence of cerebral pathology. In contrast, the presence of *T. gondii* in brains was significantly higher in cases with undernourishment (60%) than in those without undernourishment (4.3%) \( (P = 0.005) \). In total, two characteristics of cases showed \( P \) values of \( \leq 0.10 \) in the bivariate analysis: age \( (P = 0.07) \) and undernourishment \( (P = 0.005) \). Further analysis of these variables by logistic regression showed that the presence of *T. gondii* in brains was only associated with undernourishment \( \text{OR} = 33.90; 95\% \text{ CI} : 2.82 - 406.32; P = 0.005 \). The result of the Hosmer–Lemeshow test suggested an acceptable fit of our regression model \( (P = 0.45) \).

**Discussion**

The demonstration of *T. gondii* cysts in brain and heart by immunohistochemistry in autopsy series has been scantily reported. In the present study, we determined the presence of *T. gondii* in brain and heart in an autopsy series in a public general hospital in Durango City, Mexico by using immunohistochemistry. Our results showed that 9.8\% of the autopsy cases studied had *T. gondii* in their brains. In contrast, none of the heart tissues in the autopsy cases were positive for *T. gondii* by immunohistochemistry. There is poor knowledge on the frequency of *T. gondii* in differ-
ent organs and tissues in *T. gondii* infected individuals. Results suggest that *T. gondii* can be more likely found in brain than in heart of infected persons. In the present study, we were able to visualize *T. gondii* for the first time in brain of infected individuals in the region. Results add evidence to previous seroprevalence studies confirming that *T. gondii* infection occurs commonly in people in the region [22–25]. The 9.8% frequency of demonstration of *T. gondii* in brain in autopsy cases is higher than the 6.1% seroprevalence of *T. gondii* infection reported in the general population in Durango City, Mexico [22]. It is not clear why these autopsy cases had a higher frequency of *T. gondii* in their brains than the seroprevalence reported in the general population. However, there are differences in

The characteristics of the studied populations. All autopsy cases died because of a number of underlying diseases whereas subjects in the general population included both healthy and ill people. Of note, infection with *T. gondii* in autopsy cases predominated in young cases. Indeed, four (80%) of the five positive autopsy cases were younger than 30 years old. Whereas, only 20 (33.9%) of 59 positive subjects in the survey of the general population were 30 years old or younger [22]. Seroprevalence of *T. gondii* infection increases with age in the region [22, 23]. One wonders whether the inverse “prevalence” pattern observed in autopsy cases points towards a role of infection in the pathology/death of the subjects. None of the autopsy cases had clinical or postmortem diagnoses of toxoplasma.

| Characteristics          | No. of autopsy cases tested | Prevalence of infection | P value |
|--------------------------|----------------------------|-------------------------|---------|
|                          | No. | %    |       |         |
| Age                      |     |      |       |         |
| Up to 10 years           | 11  | 1    | 9.1   | 0.07    |
| 11–30 years              | 11  | 3    | 27.3  |         |
| >30 years                | 29  | 1    | 3.4   |         |
| Sex                      |     |      |       |         |
| Male                     | 24  | 2    | 8.3   | 1       |
| Female                   | 27  | 3    | 11.1  |         |
| Birthplace               |     |      |       |         |
| Durango State            | 48  | 5    | 10.4  | 0.84    |
| Other state or abroad    | 2   | 0    | 0.0   |         |
| Unknown                  | 1   | 0    | 0.0   |         |
| Residence                |     |      |       |         |
| Urban                    | 23  | 3    | 13    | 0.7     |
| Rural                    | 25  | 2    | 8     |         |
| Unknown                  | 3   | 0    | 0.0   |         |
| Education                |     |      |       |         |
| Up to 6 years            | 31  | 3    | 9.7   | 0.61    |
| >6 years                 | 14  | 2    | 14.3  |         |
| Unknown                  | 6   | 0    | 0.0   |         |
| Occupation               |     |      |       |         |
| Laborer                  | 12  | 1    | 8.3   | 0.64    |
| Nonlaborer               | 33  | 4    | 12.1  |         |
| Unknown                  | 6   | 0    | 0.0   |         |
| Pathology in brain       |     |      |       |         |
| Yes                      | 35  | 3    | 8.6   | 0.64    |
| No                       | 16  | 2    | 12.5  |         |
| Undernourishment         |     |      |       |         |
| Yes                      | 5   | 3    | 60.0  | 0.005   |
| No                       | 46  | 2    | 4.3   |         |
miosis. However, toxoplasmosis in Mexico is a neglected disease and diagnostic examinations are not routinely performed. None of the autopsy cases had serological results for *T. gondii*. The rate of *T. gondii* in brains likely is even higher than 9.8% because a negative immunohistochemistry result cannot exclude presence of cysts in the brain. In autopsy series of AIDS, researchers found *T. gondii* in brains in 15 of 70 autopsy cases in Germany [20] and in heart in 21 of 170 autopsy cases in France [21] by using immunohistochemistry. In the present study, we found *T. gondii* in the choroid plexus in an autopsy case (No. 21) with cerebral ischemia and undernourishment. Tachyzoites of *T. gondii* have been observed in the choroid plexus in 53% of patients with acquired immunodeficiency syndrome with cerebral toxoplasmosis [25]. No information about serology for human immunodeficiency virus infection was available in any of the autopsy cases in our series. We are not aware of any report of *T. gondii* in choroid plexus in immunocompetent individuals. Remarkably, in the present study, *T. gondii* infection in brain was associated with undernourishment by bivariate analysis, and this association remained significant by multivariate analysis, too. To the best of our knowledge, this is the first report of an association of *T. gondii* infection with undernourishment. It is unclear why autopsy cases with undernourishment had a higher frequency of detection of *T. gondii* than those without undernourishment. Malnutrition profoundly affects immune responses preventing the host from mounting an adequate protective response to infectious agents [26]; it remains to be shown whether malnutrition has a causal association with the dissemination of *T. gondii*. *T. gondii* is located in all brain areas [27], although some studies reported high number of parasites in the amygdala and frontal cortex [27, 28].

Conclusions

We demonstrated the presence of *T. gondii* in brains in Durango City, Mexico. Results suggest that *T. gondii* can be more readily found in brains than in hearts of infected individuals. We report for the first time an association of *T. gondii* cysts in brains with undernourishment.

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References

1. Smith JE: A ubiquitous intracellular parasite: the cellular biology of *Toxoplasma gondii*. Int J Parasitol 25, 1301–1309 (1995)
2. Hill DE, Chirukandoth S, Dubey JP: Biology and epidemiology of *Toxoplasma gondii* in man and animals. Anim Health Res Rev 6, 41–61 (2005)
3. Montoya JG, Liesenfeld O: Toxoplasmosis. Lancet 363, 1965–1976 (2004)
4. Jones JL, Dubey JP: Foodborne toxoplasmosis. Clin Infect Dis 55, 845–851 (2012)
5. Dubey JP (2010): Toxoplasmosis of Animals and Humans, 2nd ed. CRC Press, Boca Raton, Florida
6. Kamerkar S, Davis PH: *Toxoplasma* on the brain: understanding host-pathogen interactions in chronic CNS infection. J Parasitol Res 2012, (2012)
7. Blanchard N, Dunay IR, Schlüter D: Persistence of *Toxoplasma gondii* in the central nervous system: a fine-tuned balance between the parasite, the brain and the immune system. Parasite Immunol 37, 150–158 (2015)
8. Weiss LM, Dubey JP: Toxoplasmosis: a history of clinical observations. Int J Parasitol 39, 895–901 (2009)
9. Gajewski PD, Falkenstein M, Hengstler JG, Golka K: *Toxoplasma gondii* impairs memory in infected seniors. Brain Behav Immun 36, 193–199 (2014)
10. Flegr J: How and why *Toxoplasma* makes us crazy. Trends Parasitol 29, 156–163 (2013)
11. Arling TA, Yolken RH, Lapidus M, Langenberg P, Dickerson FB, Zimmerman SA, Balis T, Cabassa JA, Scandris DA, Tonelli LH, Postolache TT: *Toxoplasma gondii* anti-body titers and history of suicide attempts in patients with recurrent mood disorders. J Nerv Ment Dis 197, 905–908 (2009)
12. Alvarado-Esquível C, Sánchez-Anguiano LF, Arnaud-Gil CA, López-Longoria JC, Molina-Espinoza LF, Estrada-Martínez S, Liesenfeld O, Hernández-Tinoco J, Sifuentes-Álvarez A, Salas-Martínez C: *Toxoplasma gondii* infection and suicide attempts: a case-control study in psychiatric outpatients. J Nerv Ment Dis 201, 948–952 (2013)
13. Alvarado-Esquível C, Torres-Castorena A, Liesenfeld O, Estrada-Martínez S, Urbina-Alvarez JD: High seroprevalence of *Toxoplasma gondii* infection in a subset of Mexican patients with work accidents and low socioeconomic status. Parasit Vectors 5, 13 (2012)
14. Dixit PG, Umap PS, Bardale RV: *Toxoplasma* myocarditis presenting as myocardial infarction. Indian J Med Sci 61, 218–220 (2007)
15. Strabelli TM, Siciliano RF, Vidal Campos S, Bianchi Castelli J, Bacal F, Bocchi EA, Uip DE: *Toxoplasma gondii* myocarditis after adult heart transplantation: successful prophylaxis with pyrimethamine. J Trop Med 2012, 853562 (2012)
16. Mroczek-Czernecka D, Rostoff P, Piwowarska W: Acute toxoplasmic perimyocarditis in a 67-year-old HIV-negative woman – a case report. Przegl Lek 63, 100–103 (2006)
17. Pergola G, Cascone A, Russo M: Acute pericarditis and myocarditis by *Toxoplasma gondii* in an immunocompetent young man: a case report. Infez Med 18, 48–52 (2010)
18. Guillot JP, Beylot J, Turner K, Lacoste D, Gabinshi C, Besse P: Acute cardiac insufficiency and toxoplasmosis. Arch Mal Coeur Vaiss 82, 1767–1770 (1989)
19. Jautzke G, Sell M, Thalmann U, Janitschke K, Iglesias J, Schüermann B, Ruh F: Immunohistochemical demonstration of extracerebral toxoplasmosis in AIDS. Verh Dtsch Ges Pathol 75, 185–188 (1991)
20. Neuen-Jacob E, Figge C, Arendt G, Wendtland B, Jacob B, Wechsler W: Neuropathological studies in the brains of...
AIDS patients with opportunistic diseases. Int J Legal Med 105, 339–350 (1993)
21. Hofman P, Bernard E, Michiels JF, Thyss A, Le Fichoux Y, Loubière R: Extracerebral toxoplasmosis in the acquired immunodeficiency syndrome (AIDS). Pathol Res Pract 189, 894–901 (1993)
22. Alvarado-Esquivel C, Estrada-Martínez S, Pizarro-Villa-lobos H, Arce-Quinones M, Liesenfeld O, Dubey JP: Seroepidemiology of Toxoplasma gondii infection in general population in a northern Mexican city. J Parasitol 97, 40–43 (2011)
23. Alvarado-Esquivel C, Liesenfeld O, Burciaga-López BD, Ramos-Nevárez A, Estrada-Martínez S, Cerrillo-Soto SM, Carrete-Ramírez FA, López-Centeno Mde L, Ruiz-Martínez MM: Seroepidemiology of Toxoplasma gondii infection in elderly people in a northern Mexican city. Vector Borne Zoonotic Dis 12, 568–574 (2012)
24. Alvarado-Esquivel C, Cruz-Magallanes HM, Esquivel-Cruz R, Estrada-Martínez S, Rivas-González M, Liesenfeld O, Martinez-Garcia SA, Ramírez E, Torres-Castorena A, Castañeda A, Dubey JP: Seroepidemiology of Toxoplasma gondii infection in human adults from three rural communities in Durango State, Mexico. J Parasitol 94, 811–816 (2008)
25. Falangola MF, Petito CK: Choroid plexus infection in cerebral toxoplasmosis in AIDS patients. Neurology 43, 2035–2040 (1993)
26. Iliakis D, Kressig RW: The relationship between malnutrition and immune. Ther Umsch 71, 55–61 (2014)
27. McConkey GA, Martin HL, Bristow GC, Webster JP: Toxoplasma gondii infection and behaviour – location, location, location? J Exp Biol 216, 113–119 (2013)
28. Vyas A, Kim SK, Giacomin N, Boothroyd JC, Sapolsky RM: Behavioral changes induced by Toxoplasma infection of rodents are highly specific to aversion of cat odors. Proc Natl Acad Sci U S A 104, 6442–6447 (2007)