A large-scale study on the seroprevalence of *Toxoplasma gondii* infection in humans in Iran

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Abstract: It is estimated that nearly one-third of the world’s human population is infected with *Toxoplasma gondii* (Nicolle et Manceaux, 1908). Human infection is commonly asymptomatic, multifaceted, and can manifest in severe pathological forms in congenital toxoplasmosis and immunocompromised individuals. This study attempted to recognize the seroprevalence of *T. gondii* infection in Iranian residents referred to medical laboratories for toxoplasmosis tests throughout the country. This retrospective cross-sectional study was conducted from 2015 to 2019 on individuals referred to diagnostic laboratories in 26 provinces, and these laboratories sent their samples to the referral centres. Accordingly, data associated with serodiagnosis of toxoplasmosis, age, sex, anti-*T. gondii* IgG, and IgM status in Iranian residents were collected from two referral diagnostic laboratories. All individuals were evaluated using the antibody immunocapture-chemiluminescence assay (CLIA) method with the Immulite®2000s XPI system. In this study, the first large-scale assay of *T. gondii* infection in Iran, an overall seroprevalence of 30.4% was among 35,047 patients examined. The highest IgM seropositivity rate was in the 10–20 years old patients. In addition, this study showed a very different prevalence of *T. gondii* across the country, highest in the humid areas, such as the Caspian Sea basin in the North, and the North West with seroprevalence of 48.6%.

Key words: Prevalence, toxoplasmosis, IgG/IgM, chemiluminescence, antibody, public health

*Toxoplasma gondii* (Nicolle et Manceaux, 1908) is an obligatory apicomplexan intracellular parasite that infects about a third of humans worldwide and can infect all warm-blooded animals (Molan et al. 2019). Felids are definitive hosts for this parasite, shedding up to millions of oocysts per day in their faeces, which sporulate and become infective in the environment. Ingestion of these sporulated oocysts, which contaminate vegetables, soil and water sources, together with eating bradyzoites from raw or undercooked meat, comprise the two major horizontal transmission routes (Hill and Dubey 2018, Attias et al. 2020). Indeed, these parasite stages are responsible for a substantial burden of postnatally acquired infections, potentially causing sporadic outbreaks of acute or mild disease in immunocompetent people and severe toxoplasmosis in immunocompromised individuals, including HIV/AIDS patients, and cases of congenital infection during pregnancy, following reactivation of bradyzoites into disseminating tachyzoites (Nayeri et al. 2020, Thebault et al. 2021).

Infection with *T. gondii* in pregnancy is associated with foetal loss and foetal and newborn abnormalities in humans and livestock. This parasite also causes infections in the eye (retina) and brain, necessitating the use of drugs that cross the placenta, brain and eye barriers for penetration (Aguirre et al. 2019). In addition, given the growing number of cancer and immunocompromised patients and organ transplant recipients, *T. gondii*, as an opportunistic parasite, can drive acute life-threatening conditions for those patients (Aguirre et al. 2019). Owing to the tropism of *T. gondii* for the central nervous system (CNS) and eye, poor prognosis and complications such as glaucoma, chorioretinitis, retinal detachment, brain abscess and encephalitis can occur during acute or recrudescent infection (Weiss and Dubey 2009, Kalogeropoulos et al. 2021). Furthermore, congenital toxoplasmosis may cause serious complications such as focal brain lesions, microcephaly, hydrocephaly, deafness and mental retardation (Daher et al. 2021). Cerebral toxoplasmosis may also cause neuropsychological effects, such as obsessive-compulsive.

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Several factors such as parasite strains, number of ingested organisms, genetic background, immunity status, and sex of the host are crucial in the progression of infection (Montoya and Liesenfeld 2004). Infection with *T. gondii* has severe socioeconomic effects on individuals. Families incur many costs while caring for sick children, especially those with mental retardation and blindness (Roberts et al. 1994, Ben-Harari and Connolly 2019).

In highly endemic areas, such as parts of Africa and continental Europe and Central and South America, seroprevalence can reach around 90%, whereas in some European areas it can reach 60%, and ranges from 8 to 22% in the United States (Dubey and Jones 2008, Aguirre et al. 2019, Molan et al. 2019). The prevalence of the infection fluctuates depending on the region, climate, diet, hygiene and host susceptibility (Almeria et al. 2021).

In Iran, several studies with various serological methods were performed on different human populations (Nayeri et al. 2020, Sobati 2020), particularly high-risk demographic groups, such as people undergoing chemotherapy (Hosseini et al. 2021), ophthalmic disorders (Saki et al. 2020), transplant, immunocompromised and hemodialysis patients (Ahmadpour et al. 2014, Rasti et al. 2016), diabetic patients (Asgari et al. 2021), pregnant women (Ben-Harari and Connolly 2019, Khademi et al. 2019, Nayeri et al. 2020), neonates (Nahvi et al. 2019), blood donors (Moshfe et al. 2018), and occasionally healthy human population (Mousavi-Hasanzadeh et al. 2020). The seroprevalence ranged from 13% to 73% and varied among gender, province and lifestyle (Daryani et al. 2014).

As a cosmopolitan strategy, the One Health concept has emphasised the need for an interdisciplinary effort to seek optimal human, animal and ecosystem health. This approach suggested zoonotic infection monitoring for *T. gondii* prevalence by integrating data on humans, domestic animals and wildlife in the same area for better management (Aguirre et al. 2019). Standard diagnostic methods for toxoplasmosis generally pertain to immunology-dependent examinations such as enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence assay (IFA) to detect the *T. gondii*-specific antibodies, i.e., IgM or IgG (Robert-Gaingneux and Darde 2012).

Numerous seroprevalence studies show that the different results reported from a particular area can vary due to the selected target human population and the applied laboratory methods and diagnostic kits (Daryani et al. 2014, Khademi et al. 2019, Saki et al. 2020). Therefore, restricting these variables can better express the existing real situation. In Iran, serological and immunological tests in diagnostic laboratories are usually performed by kits with varying characteristics and performance levels on the Iranian market. To the best of our knowledge, this study is the first extensive and non-local evaluation of the *T. gondii* seroprevalence in Iran with the susceptible and specific reliable technique allowing for comfort and safety in serological interpretation.

In this study, we determined the seroprevalence of *T. gondii* based on the test performed previously using *T. gondii* Quantitative IgG/IgM Kits (Siemens Healthcare Diagnostics Products, Marburg, Germany) with an automated assay Immulite® 2000s analyser (Euro/Diagnostic Products, Marburg, Germany) and indirect immunofluorescence assay (IFA) to detect the *T. gondii*-specific antibodies, i.e., IgM or IgG (Robert-Gaingneux and Darde 2012).

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with a single sensitive technique (antibody immunocapture-chemiluminescence assay).

MATERIALS AND METHODS

Study design and sample collection

A large-scale retrospective cross-sectional study was carried out in 26 of 31 provinces in Iran – East Azerbaijan, Zanjan, Ardabil, Gilan, Mazandaran, Golestan, Qazvin, Alborz, Tehran, Semnan, Markazi, Ilam, Kurdistan, Hamedan, Lorestan, Qom, Isfahan, Yazd, Kohgiluyeh and Boyer-Ahmad, Fars, Kerman, Sistan and Balochistan, Hormozgan, Khuzestan and Bushehr – between September 2015 and March 2020. To diagnose toxoplasmosis, the recorded data of the 35,047 sera samples of the human population, including 16,999 anti- \textit{T. gondii} IgM and 18,048 anti- \textit{T. gondii} IgG tests. Medical Laboratories Noor Lab in Tehran and Pooyesh Lab in Isfahan, Iran, processed the samples in a determined way and used \textit{Toxoplasma gondii} Quantitative IgG/IgM kits (Siemens Healthcare Diagnostics Products, Marburg, Germany) in the following section. About three to five ml of blood samples were obtained from the median cubital and cephalic veins. Sera were separated from cell pellets in a different tube to limit the interference of fibrin that may lead to false-positive results using automated immunoassay. The individuals were classified into ten age groups: < 1 month, < 1 year, 1–10 years, 10–20 years, 20–30 years, 30–40 years, 40–50 years, 50–60 years, 60–70 years, > 70 years (Table 1).

Diagnostic analysis

According to the manufacturer’s instructions, DPC Immulite\textsuperscript{\textregistered}2000s \textit{Toxoplasma} Quantitative IgG/IgM Kits were used. Antibody immunocapture-chemiluminescence assay (CLIA) method with Immulite\textsuperscript{\textregistered}2000s XPi System (Euro/Diagnostic Products Corporation, Siemens Medical, Gwynedd, UK) was used. The positivity threshold with the Immulite\textsuperscript{\textregistered}2000s technique was considered 8 IU/ml, with an uncertain and equivocal area from 6.5 to 8 IU/ml, and below 6.5 IU/ml deemed negative.

Statistical analysis

IBM SPSS 22.0 software (Armonk, New York, USA) was used for statistical analyses. The prevalence of IgG and IgM antibodies against \textit{T. gondii} was estimated from the ratio of positive samples to the total number of samples tested, with a 95% confidence interval (CI). Associations between \textit{T. gondii} seroprevalence and the explanatory variables (age, sex and sampling area) were initially screened using the Chi-square test or Fisher’s test. All statistical variables with a \(p\)-value < 0.05 were considered significant. A geographic information system (GIS) map was created for anti- \textit{T. gondii} IgM and IgG seropositivity for studied provinces of the country, using ESRI ArcGIS 10.4 software (Esri, Redlands, California).

RESULTS

In total, 35,047 samples of human sera were referred to Medical Laboratories for \textit{T. gondii} IgG and IgM tests, 87.45% (\(n = 30,649\)) tests were related to females, and 12.54% (\(n = 4,398\)) tests were for males (Table 1). The mean age of the studied patients was 29.2 years (from 1 day up to 99 years old; median 30 years). The mean age of anti- \textit{T. gondii} IgM seropositive patients was significantly lower (25.9 years) than that of negative patients (29.5 years) (\(p < 0.001\)). In people who were anti- \textit{T. gondii} IgG seropositive, the mean age was significantly higher (32.1 years) than
the mean age in negative patients (27.6 years) \((p < 0.01)\). The anti-\textit{T. gondii} IgG seropositivity showed a high prevalence in neonates < 1-month-old (38.5%) and < 1-year-old (33.7%) and then declined to 9.0% and continued with a significant increase by age \((p < 0.001)\). However, considering IgM, it starts with low seropositivity in neonates < 1-month-old (1.7%) and < 1-year-old (1.3%) and a continuous with a significant increase \((p < 0.001)\) until 10–20 years old patients, then it shows a mild decrease in seropositivity by the increasing age (Fig. 1; Table 1).

The results showed that 250 (1.5%) out of 16,999 and 5,482 (30.4%) out of 18,048 individuals were anti-\textit{T. gondii} IgM and IgG seropositive, respectively. There was no significant relationship between sex and IgG seropositivity \((p = 0.094)\). The risk of infection in men compared to women was 1.055, which shows an almost equal risk for both sexes. However, the prevalence was significantly higher in men with IgM seropositivity \((p < 0.001)\), as well as the risk of infection (IgM) in men was 3.23 × higher than in women (Table 1; Fig. 2).

Since the majority of the studied population were women of childbearing ages, we divided the ages into some age groups, and the results of IgG and IgM seropositivity in females were as follows: anti-\textit{T. gondii} IgG seropositivity in < 1-year-old 34.1%, 1–10 years 10.5%, 10–20 years 20.5%, 20–30 years 25%, 30–40 years 32.8%, 40–50 years 46.1%, 50–60 years 53.9%, 60–70 years 62%, and > 70 years 71.2%. Anti-\textit{T. gondii} IgM seropositivity in < 1-year-old 2.1%, 1–10 years 1.2%, 10–20 years 3.2%, 20–30 years 1.3%, 30–40 years 0.7%, 40–50 years 1.3%, 50–60 years 1.9%, 60–70 years 2.2%, and >70 years 0%. Values of the overall prevalence of \textit{T. gondii} antibodies in different age groups are in Table 1.

The highest number of studied samples was from Tehran province, and the lowest number was from Hormozgan province (Table 2). The association between the seroprevalence and the geographic location was statistically significant \((p < 0.001)\). The highest prevalence of anti-\textit{T. gondii} IgM was observed in Kohgiluyeh and Boyer-Ahmad (5.3%), and the lowest rate in Bushehr, Zanjan, Semnan, Sistan and Baluchestan, Kerman, Kermanshah, Markazi, and Hormozgan provinces (0%) (Fig. 2). The highest prevalence of anti-\textit{Toxoplasma} IgG was found in East Azerbaijan and Mazandaran (48.6%), and the lowest rate was in samples from Sistan and Baluchistan provinces (10.6%) (Fig. 3).

**DISCUSSION**

Understanding the status of infection with \textit{Toxoplasma gondii} in each community’s population is critical (Dubey and Jones 2008). Most epidemiological surveys in Iran are based on IgG titres and the ELISA method (Daryani et al. 2014, Foroutan et al. 2019). There is limited knowledge of
the seroepidemiology of *T. gondii* in the human population of Iran, and most of the research was conducted on high-risk groups at a local level (Ahmadpour et al. 2014, Saki et al. 2020).

The major limitations of other studies were avoided in this study, such as the absence of studies in some regions of Iran and different methods without similar sensitivities and specificities (Foroutan et al. 2019). The present retrospective cross-sectional survey estimated the seroprevalence of *T. gondii* infection in the Iranian population referred to medical laboratories for *T. gondii* infection tests, using a large number of sera samples over five years.

IgG-positive-antibody titres can be detected 2–3 weeks after infection, reaching a maximum titre within two months. It then diminishes to a baseline level that persists throughout life. In contrast, IgM titres appear within a few days after infection and reach a maximum level within four weeks. It is necessary to detect both the IgG and IgM antibodies simultaneously to establish a chronic or acute phase of infection (Robert-Gangneux and Darde 2012).

Our results estimate that the seroprevalence of *T. gondii* infection in the human population of Iran is 30.4%. In other studies in Iran, slightly higher seroprevalence rates of 33–46% and 38–47% have been reported for *T. gondii* infection (Daryani et al. 2014, Foroutan et al. 2019). The difference in infection rate may be related to the study populations.

In this research, the target population was humans requested for anti-*T. gondii* IgG and IgM. The prevalence is lower in the USA/Canada, Europe, and China, being 17.5%, 29.6%, and 16.4%, respectively, primarily due to the good hygienic conditions, successful learning and cultural plans to alert people, precise diagnostic tests for women of childbearing age and pregnant women, industrialisation of animal husbandry as well as well-established monitoring systems and enhanced laws in meat inspection. In African countries, a 61.4% prevalence of *T. gondii* infection has been observed, which may be settled by the abundance of infected cats and food animals, low levels of hygiene, and living in sub-standard conditions (Molan et al. 2019).

In our work, a considerable number of the studied population were women, especially between 20 and 40 years old (women of childbearing age), and infants tested for screening, monitoring or diagnosis of toxoplasmosis. The IgG seropositivity for women in the 20–30 and 30–40 age groups was registered as 25.0% and 32.8%, respectively, and for the IgM as 1.3% and 0.7%, respectively. Notably, we observed the highest IgM seropositivity in the females in the 10–20 year-old age group (3.2%), which is very im-

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**Fig. 3.** A GIS map of IgG seroprevalence of *Toxoplasma gondii* (Nicolle et Manceaux, 1908) in different provinces of Iran, during 2015–2020.
IgG positive n (%)  67  32  18  3  19 (16)
IgG negative n (%)  1 (0.0)  1  4 (9)  42 (91)  46
Total  116 (84)  16,749

1. IgM positive n (%)  294  96 (9)  2 (10)  7  149
IgM negative n (%)  6 (15)  13 (14)  1  8 (9)  394
Total  87  4 (44)
2. Female  20  509  1 (50)  -  22 (73)
3. Male  432 (91)  87  916 (9)  1 (33)  14 (13)
Total  476  4 (67)

4. Total  294  703  2 (5)  44 (9)  3 (15)

5. X

6. Seroprevalence of human toxoplasmosis in Iran

Table 2. The prevalence of anti-Toxoplasma IgG and IgM in males and females referred from different provinces in Iran.

<table>
<thead>
<tr>
<th>Province</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
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<th>Female</th>
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</tr>
</thead>
<tbody>
<tr>
<td>East Azerbaijan</td>
<td>3</td>
<td>15</td>
<td>18</td>
<td>2</td>
<td>11</td>
<td>17 (90)</td>
<td>17 (90)</td>
<td>9</td>
<td>9</td>
<td>8</td>
<td>22</td>
<td>29 (78)</td>
</tr>
<tr>
<td>Zanjan</td>
<td>3</td>
<td>21</td>
<td>24</td>
<td>4</td>
<td>17</td>
<td>20 (83)</td>
<td>20 (83)</td>
<td>24</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Ardabil</td>
<td>9</td>
<td>28</td>
<td>37</td>
<td>23</td>
<td>72</td>
<td>75 (91)</td>
<td>75 (91)</td>
<td>13</td>
<td>14</td>
<td>27</td>
<td>50 (88)</td>
<td>50 (88)</td>
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<tr>
<td>Gilan</td>
<td>26</td>
<td>21</td>
<td>47</td>
<td>101</td>
<td>101</td>
<td>195 (81)</td>
<td>195 (81)</td>
<td>242</td>
<td>1 (33)</td>
<td>2 (67)</td>
<td>3</td>
<td>60 (19)</td>
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<tr>
<td>Mazandaran</td>
<td>44</td>
<td>11</td>
<td>55</td>
<td>368</td>
<td>89</td>
<td>412 (86)</td>
<td>412 (86)</td>
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<td>4 (22)</td>
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<tr>
<td>Golestan</td>
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<td>4</td>
<td>34</td>
<td>97</td>
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<td>35 (70)</td>
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<td>1 (33)</td>
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<td>56 (88)</td>
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<td>1 (50)</td>
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<td>194 (91)</td>
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<td>740</td>
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<td>82 (75)</td>
<td>112</td>
<td>916 (9)</td>
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<tr>
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<td>960</td>
<td>2 (5)</td>
<td>44 (9)</td>
<td>3 (15)</td>
<td>11 (79)</td>
<td>23 (72)</td>
<td>1 (79)</td>
<td>2 (3)</td>
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<td>916</td>
<td>9 (77)</td>
<td>1,333</td>
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<td>2 (67)</td>
<td>3</td>
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<td>425</td>
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<td>457 (92)</td>
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<td>144</td>
<td>249 (85)</td>
<td>66 (22)</td>
<td>66 (16)</td>
<td>-</td>
</tr>
</tbody>
</table>

| East Azerbaijan   | 20   | 509    | 529   | 1 (50)| -      | -     | -      | -      | -      | -     | -      | -     |
| Zanjan            | 4    | 14     | 18    | 50   | 97     | 57 (88)| 57 (88)| 4      | 1 (16)| 1 (16)| 2     | 11 (8)| 133 (92)|
| Ardabil           | 6     | 24     | 30    | 44   | 97     | 7 (99)| 7 (99)| 1      | 1 (84)| 1 (84)| 2     | 11 (8)| 133 (92)|
| Gilan             | 21   | 98     | 121   | 21    | 91     | 113 (99)| 113 (99)| 242    | 1 (33)| 2 (67)| 3     | 60 (19)| 261 (81)|
| Mazandaran        | 3     | 11     | 14    | 368  | 89     | 412 (86)| 412 (86)| 435    | 4 (22)| 14 (78)| 18     | 96 (9)| 922 (91)|
| Golestan          | 1     | 3      | 4     | 34   | 97     | 35 (70)| 35 (70)| 5      | 2 (67)| 1 (33)| 3     | 2 (3)| 56 (97) |
| Qazvin            | 10    | 29     | 39    | 25   | 71     | 56 (88)| 56 (88)| 1      | 1 (50)| 1 (50)| 2     | 11 (8)| 133 (92)|
| Alborz            | 17    | 9      | 26    | 177  | 91     | 194 (91)| 194 (91)| 476    | 5 (21)| 19 (79)| 24     | 55 (6)| 890 (94)|
| Tehran            | 380   | 14     | 394   | 2,403| 66     | 2,783 | 2,783| 740    | 10 (72)| 82 (75)| 112    | 916 (9)| 8,887 (91)|
| Total             | 294   | 703    | 960   | 2 (5) | 44 (9) | 3 (15) | 11 (79)| 23 (72)| 1 (79)| 2 (3)| 0 (0) | 111 (85)| 1,018 |

In reality, any meteorological factor, like suitable humidity, that can help the sporulation of oocysts of T. gondii shed in the cat faeces would occasion prolonged survival and the spread of infective parasites in the environment.
increasing the possibility of human and animal infection (Attias et al. 2020). The provinces with seroprevalence of toxoplasmosis that differed significantly from previous assessments were: East Azerbaijan, Ardabil, Gilan, Golestan, Qazvin, Tehran, Kermanshah, Isfahan, Kohgiluyeh and Boyer-Ahmad, Fars, Kerman, Sistan and Balochistan, and Khuzestan, respectively, in the study of Daryani et al. 2014. Due to economic problems, migration from rural to urban areas has been reversed in recent years.

However, migration from neighbouring countries with different climates and health conditions and cooking due to war and insecurity can complicate our assessment of metropolitan areas because IgG antibody titres persist throughout one’s life. However, there was no significant difference between the sera prevalence of men and women in this evaluation, as in many studies (Daryani et al. 2014).

To the best of our knowledge, this is the first large-scale seroprevalence study that provides a comprehensive view of the seroepidemiology of *T. gondii* in the Iranian population (from 26 of 31 provinces). In conclusion, an overall seroprevalence of 30.4% was detected. Thus, about one-third of Iranian people have been infected with *T. gondii*. Accordingly, two-thirds of the human population is seropositive and susceptible to infection. Therefore, individuals at high risk should be monitored. Several measures will aid in the prevention and control of *T. gondii* infection. These include health policies targeting the promotion of public educational programs, training courses for childbearing age and pregnant women, an inspection of food production industries, improvement of hygienic standards of abattoirs, and sanitary disposal of viscera.

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**Author Contributions.** RK: The design of the study, data collection and write-up. ME: Performed research and contributed to 1st draft. RJ: Analysis and interpretation of data. HM-E and HY-D: Supervised the research, supported this study’s implementation, provided feedback during the write-up, and final approval of the version to be submitted. All the authors read and gave final approval of the version to be submitted.

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