Evolution of Zika prevalence in a dengue hyperendemic municipality in Southern Mexico after the outbreak of 2015 to 2017

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Abstract

Objective. Estimate the Zika prevalence in a dengue-endemic municipality in Mexico, after the outbreak of 2015 to 2017. **Materials and methods.** Three serosurveys were conducted in Tapachula, Chiapas, in September 2018, March 2019 and November 2019. A commercial ZIKV and DENV anti-NS1 IgG ELISA were used to estimate each prevalence, their performance and adjustment of the cut-off value were compared with an in-house DENVs and ZIKV anti-EDIII IgG ELISA and the microneutralization test. **Results.** The anti-NS1 ZIKV titers decreased over time, causing that Zika

Resumen

Objetivo. Estimar la prevalencia de Zika en un municipio endémico de dengue en México, después del brote de 2015 a 2017. **Material y métodos.** Se realizaron tres encuestas serológicas seriadas en Tapachula, Chiapas, en septiembre 2018, marzo 2019 y noviembre 2019. Las ELISA comerciales de IgG anti-NS1 de DENV y ZIKV fueron utilizadas para estimar cada prevalencia; su desempeño y ajuste del valor de corte fueron comparadas con un ELISA casero de IgG anti-EDIII de DENV y ZIKV y la prueba de microneutralización. **Resultados.** Los títulos de anti-NS1 de ZIKV disminuyeron

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prevalence decreased from 78.02 to 45.22%, while anti-NS1 DENV titers increased, and the prevalence remained above 95% over a two-year period. **Conclusion.** Optimal Zika-prevalence estimates can be obtained in a two-years period after outbreaks in dengue-endemic areas. The extension of the Zika outbreak is significantly higher than previously reported in Tapachula, highlighting the underreport of cases based on the routine flavivirus surveillance system in Mexico.

Keywords: Zika seroprevalence; dengue-endemic area; surveillance; population-based serosurveys

a lo largo del tiempo, haciendo que la prevalencia de Zika disminuyera de 78.02 a 45.22%; los títulos de IgG anti-NS1 de DENV incrementaron y la prevalencia permaneció por encima de 95% en un periodo de dos años. **Conclusión.** Estimaciones óptimas de la prevalencia de Zika pueden ser obtenidas en un periodo de dos años después del brote en un área endémica de dengue. La extensión del brote de Zika es significativamente más alta que la previamente reportada en Tapachula, Chiapas, resaltando el subreporte de casos basado en el sistema rutinario de vigilancia de flavivirus en México.

Palabras clave: seroprevalencia de Zika; área endémica de dengue; vigilancia; encuestas serológicas de base poblacional

The Zika virus (ZIKV) produced extensive outbreaks in the last decades[1,2] including a large epidemic across Latin America (2015-2017).[3] This outbreak was facilitated by the extensive dissemination of the mosquito vectors, *Aedes aegypti* and *Ae. albopictus*,[4] which also transmit dengue viruses. The association of ZIKV infections with Guillain-Barré Syndrome in adults,[5,6] miscarriages, and birth anomalies during pregnancy,[7] increases the concern for future outbreaks.

The national epidemiological surveillance of Mexico reported 11 667 confirmed cases of Zika during the outbreak (2015-2017),[8] but the real magnitude of the epidemic was underestimated due to the high proportion of clinical mild cases and asymptomatic infections,[9,10] and the similarity of its clinical manifestations with dengue.[11] Another contributing factor was that confirmatory molecular testing (RT-PCR) is only useful during the first 5-7 days after the onset of the symptoms and is not widely available across regions.[12]

Population-based serosurveys provide an accurate prevalence estimator to understand the real magnitude of epidemics and possibly contribute to the identification of at-risk populations.[13] However, in dengue-endemic regions, Zika seroprevalence estimations are difficult due to the extensive cross-reactivity of the elicited antibodies in both diseases.[14,15] Studies of the first Zika epidemics used seroprevalence of IgG against the NS1 Zika protein using enzyme-linked immunosorbent assays (ELISA).[1,2] The commercial tests demonstrated high diagnostic performance in dengue non-endemic populations.[16] As the Zika epidemic advanced in dengue-hyperendemic areas, most of these ELISA depicted low specificity.[17,18] Other techniques are also affected, including neutralization tests, which is considered the gold standard test in flavivirus infections.[19] Conversely, the ELISA based on the domain III of E protein (EDIII) reported higher specificity in flavivirus-endemic populations,[20] although there are no commercially available anti-EDIII IgG ELISAs.

In order to describe the extension of the Zika outbreak in concurrence with dengue, we estimate the Zika and dengue seroprevalence in a Mexican hyper-endemic dengue municipality, prior evaluation and adjustment of serological tests performance to correct the possible biases due to cross-reactive antibodies. We also explore sociodemographic characteristics associated with Zika positivity to target at risk subpopulations.

Materials and methods

Study design

Three cross-sectional population-based serosurveys were performed sequentially during September 2018, March 2019, and November 2019. Tapachula City was chosen as the study site due to historical high dengue incidence, *i.e.*, more than 5 000 cases reported during a 10-year period (2008-2017), and Zika cases reported during the outbreak (772 confirmed cases).[21] Tapachula is a border city of Chiapas State purportedly the entrance of Chikungunya and Zika epidemics to Mexico.[22]

Basic geostatistical areas (AGEBs, in Spanish) were used to define the study area. Fifty-three AGEBs were selected in consultation with the Jurisdictional authorities using the 2010 National Cartography published by the *Instituto Nacional de Estadística y Geografía* (Inegi, in Spanish). A target sample size of 250 participants was calculated to estimate an incidence of at least 30% with 3% precision and 95% confidence level. Assuming a refusal rate of 15%, the target enrollment was 280 participants. Households within each AGEB were chosen by random sampling proportional to the sample size.

A person older than two years in each household was selected to provide a blood sample and answer a questionnaire (legal guardians answered the questionnaires for minors). The questionnaire included sociodemographic characteristics, housing quality, customary

activities, and mobility. It also ascertained self-reported history of dengue and Zika at any time, and symptoms compatible with the acute phase of both diseases. Different individuals were included in each serosurvey.

Laboratory testing

Each participant provided a 5-mL blood sample collected by venipuncture in dry sterile tubes (Vacutainer No Additive. Becton-Dickinson, Inc.; Franklin Lakes, NJ, USA). Blood samples were stored at 4°C in portable coolers until processed at the jurisdictional laboratories of Tapachula Public Health System. Blood samples were centrifuged for 10 minutes at 3 500 rpm to obtain serum and maintained at -70ºC until processed at *Instituto Nacional de Salud Pública* (INSP, in Spanish).

Adjustment serological test using control sera. Commercial dengue virus (DENV) and ZIKV anti-NS1 IgG ELISAs (Euroimmun, Lübeck, Germany) were assessed in parallel to determine the dengue and Zika prevalence. The panel of control sera and the diagnostic performance of the tests are described in appendix S1.[23] DENV and ZIKV anti-NS1 IgG titers were expressed in relative units (RU/mL). Samples above 20 RU/mL were considered positive for dengue or Zika according to the manufacturer's recommendations.[24]

The Zika commercial test was recalibrated to 130 RU/ml to improve the specificity. An *in-house* anti-EDIII-based IgG ELISA[25] and microneutralization test (MNT)[26] were used to compare the effect of the cross-reactivity in the Zika-test specificity.[23]

Statistics analysis

To evaluate diagnostic performance, we estimated the specificity (Sp), sensitivity (Se), positive predictive value (PPV), and negative predictive value (NPV) of each test using positive and negative controls. The VPP and NPV were estimated with 60% expected prevalence of dengue and 16% of Zika. We reported Zika and dengue prevalence with 95% confidence intervals (95%CI) adjusted by each test performance as indicated in appendix S1.[23,27,28] U Mann-Whitney test was used to evaluate Zika titers between serosurveys and by sociodemographic characteristics. To explore associated covariates to Zika positivity, we fitted a multivariable logistic model with the sociodemographic characteristics and Zika positivity as the outcome. We used Stata, version 14* and GraphPad PRISMA version 8 (San Diego, CA).

* StataCorp. Stata Stadistical Software 14. Collage Station, TX: StataCorp LLC, 2015.

Ethics statement

This project was approved by ethic, research and biosecurity Committee of the INSP with numbers 1141, CI-705-2020 and CB20-219, respectively. In turn, this project belonged to protocol number 279079, approved by INSP's research Committee (CI-776-2017). Written consent to participate was obtained from all adult participants and emancipated minors; parental written consent and participant assent were obtained for children.

Results

Evaluation and adjustment of the serological test

The commercial DENV anti-NS1 IgG ELISA showed 100% (95%CI: 100,100) sensitivity and 87.5% (95%CI: 73.68,100) specificity; PPV was 93.33% (95%CI: 82.91,100) and NPV was 100% (95%CI: 100,100) (table I). The commercial ZIKV anti-NS1 IgG ELISA sensitivity was 83.33% (95%CI: 71.16,95.51) (table I). A perfect specificity was found in absence of dengue exposure, the test lost specificity as dengue seroprevalence increased: 93.40% (95%CI: 86.20,97.54) in dengue seroprevalence of 20.87% (95%CI: 12.5,29.3) and, 43.33% (95%CI: 27.15,59.52) in a dengue seroprevalence of 83.39% (95%CI: 79.05,87.72). With the recalibrated cutoff value (130 RU/mL), the test reached a sensitivity of 83.33% and specificity of 90%; and PPV and NPV of 62.50 and 96.46%, respectively (table I).

The specificity obtained by the recalibration method was higher than the obtained in ZIKV-MNT, which showed 66.67% (95%CI: 47.19,82.71) in a dengue seroprevalence of 100% (95%CI: 100,100). The in-house ZIKV anti-EDIII IgG ELISA showed a perfect specificity in a dengue seroprevalence of 53.33% (95%CI: 40.00,63.33) (table I). Zika and dengue prevalence were estimated using ZIKV and DENV commercial tests.

Serosurveys

The first sampling took place in September 2018 and included 305 participants; the second collection included 278 individuals in March 2019; and the third one included 284 individuals in November 2019. In the three serosurveys, more than 70% of participants were women and 50% were housewives; close to 90% were adults, and 30% had elementary or less education. Households with low income, households with discontinued water supply, and households with stored water on average represented 55, 33, and 30.4%, respectively (table II). No symptomatic arbovirosis cases were found.

Table I

COMPARATIVE DIAGNOSTIC PERFORMANCE OF THREE SEROLOGICAL TESTS EVALUATED IN A PANEL OF CONTROLS SERA FROM DENGUE HYPER-ENDEMIC MUNICIPALITY COLLECTED BEFORE AND DURING ZIKA OUTBREAK. TAPACHULA, CHIAPAS, 2018-2019

Parameter	*MNT*		*Anti-EDIII IgG ELISA*		*Anti-NS1 IgG ELISA before re-calibration (20 RU/mL)*		*Anti-NS1 IgG ELISA after re-calibration (130 RU/mL)*	
	%	*95%CI*	*%*	*95%CI*	*%*	*95%CI*	*%*	*95%CI*
Sensitivity	--	--	81.25	69.97,92.53	83.33	71.16,95.51	83.33	71.16,95.51
Specificity	66.67	47.19, 82.71	100	100,100	43.33	27.15,59.52	90.00	80.20,99.80
PPV	--	--	100	100,100	22.73	9.04,36.42	62.50	46.69,78.31
NPV	--	--	90.91	82.60,99.22	92.86	84.44,100	96.43	90.37,100

MNT: microneutralization test; PPV: positive predictive value; NPV: negative predictive value; 95%CI: 95% confidence intervals.
Note: PPV and NPV were estimated with 16% expected Zika prevalence.

Table II

SOCIODEMOGRAPHIC CHARACTERISTICS OF SEROSURVEYS. TAPACHULA, CHIAPAS, 2018-2019

Sociodemographic characteristics	*Tapachula (Sept, 2018)*			*Tapachula (Mar, 2019)*			*Tapachula (Nov, 2019)*		
	N	*%*	*95%CI*	*N*	*%*	*95%CI*	*N*	*%*	*95%CI*
Total	305	100.0		280	100.0		285	100.0	
Sex									
Male	81	26.6	22.0,31.1	79	28.4	23.7,33.1	62	21.8	17.5,26.1
Female	224	73.4	68.8,78.0	199	71.6	66.9,76.3	183	64.4	59.5,69.4
Age (years)									
01-17	27	8.9	5.9,11.8	9	3.2	1.4,5.1	20	7.0	4.4,9.7
18-29	62	20.3	16.1,24.5	47	16.9	13.0,20.8	51	18.0	14.0,21.9
30-39	42	13.8	10.2,17.4	41	14.7	11.0,18.4	52	18.3	14.3,22.3
40-49	71	23.3	18.9,27.7	51	18.3	14.3,22.4	48	16.9	13.0,20.8
50-59	55	18.0	14.0,22.0	51	18.3	14.3,22.4	45	15.8	12.1,19.6
60-100	48	15.7	12.0,19.5	79	28.4	23.7,33.1	68	23.9	19.5,28.4
Education									
Illiteracy	38	12.5	9.0,15.9	32	11.5	8.2,14.8	36	12.7	9.2,16.1
Elementary school	80	26.2	21.7,30.8	57	20.5	16.3,24.7	87	30.6	25.8,35.4
Middle school	93	30.5	25.7,35.3	73	26.3	21.7,30.8	84	29.6	24.8,34.3
High school	57	18.7	14.6,22.7	70	25.2	20.7,29.7	48	16.9	13.0,20.8
University or postgrad	36	11.8	8.4,15.2	47	16.9	13.0,20.8	29	10.2	7.1,13.4
Occupation									
Student	27	8.9	5.9,11.8	23	8.3	5.4,11.1	20	7.0	4.4,9.7
Merchant	30	9.8	6.7,12.9	29	10.4	7.2,13.6	19	6.7	4.1,9.3
Unemployed	10	3.3	1.4,5.1	4	1.4	0.2,2.7	13	4.6	2.4,6.7

(continues…)

(continuation)

Manual worker	45	14.8	11.1,18.4	70	25.2	20.7,29.7	47	16.5	12.7,20.4
Households worker	165	54.1	48.9,59.3	130	46.8	41.6,51.9	170	59.9	54.8,65.0
Professional	27	8.9	5.9,11.8	23	8.3	5.4,11.1	15	5.3	3.0,7.6
Households incomes ($)									
≥4 501	44	14.4	10.8,18.1	24	8.6	5.7,11.5	38	13.4	9.8,16.9
<1 500	143	46.9	41.7,52.1	97	34.9	29.9,39.8	8	2.8	1.1,4.5
1 501-4 500	118	38.7	33.6,43.7	65	23.4	18.9,27.8	58	20.4	16.2,24.6
Run water availability									
Yes	135	44.3	39.1,49.4	194	69.8	65.0,74.6	245	86.3	82.7,89.8
No	170	55.7	50.6,60.9	85	30.6	25.8,35.4	36	12.7	9.2,16.1
Domestic stored water									
No	161	52.8	47.6,58.0	244	87.8	84.4,91.2	193	68.0	63.1,72.8
Yes	144	47.2	42.0,52.4	34	12.2	8.8,15.6	90	31.7	26.8,36.5

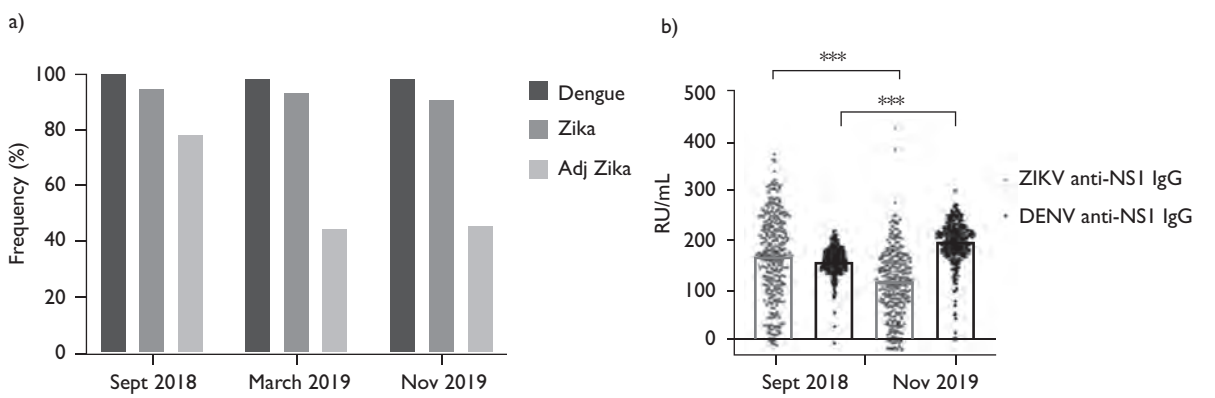
95%CI: 95% confidence intervals

Zika prevalence

Adjusted dengue prevalence was above 95% with low variability across serosurveys. The crude Zika seroprevalence was above 90% across the three serosurveys. However, the adjusted Zika prevalence showed a notable decline along the three serosurveys from 78.02% (95%CI: 45.33,100) in September 2018 to 43.83% (95%CI: 22.68,64.98) in March 2019, and to 45.22% (95%CI: 23.68, 66.76) in November 2019 (figure 1a).

The mean of the ZIKV anti-NS1 IgG titers dropped between the first and third serosurveys from 168.5 to 116.0 RU/mL ($p<0.0001$) (figure 1b). Interestingly, titers did not decrease in participants older than 50 years. In contrast, the mean of the DENV anti-NS1 IgG titers increased from 157.0 to 195.8 RU/mL ($p<0.0001$) in all age groups (figure 1b).

Our estimates of seroprevalence sharply contrast with the small number of cumulative confirmed Zika infections reported in Tapachula (156 cases), Chiapas (772 cases) and Mexico (11 667 cases) during the outbreak.[8] The estimated prevalence would translate into 159 946 cases in Tapachula that is 1 000, 207 and 13 times more than the total number of the confirmed cases



a) Zika prevalence in Tapachula one and two years after Zika outbreak in Mexico. Dengue (black bar) and Zika (light gray bar) prevalence were estimated using anti-NS1 IgG ELISA according to cutoff value recommended by manufacturer (20 RU/mL). Adjusted Zika prevalence (dark gray bar) was estimated using the new cutoff value (130 RU/mL). b) The mean of the ZIKV (gray points) and DENV (black points) anti-NS1 IgG titers in the first (September 2018) and third (November 2019) serosurveys. The statistical significance is indicated with asterisks ($p<0.001$); Adj Zika: Adjusted Zika; ZIKV: Zika virus; DENV: dengue virus; RU: relative units

Figure 1. Evolution of Zika prevalence in Tapachula, Chiapas, 2018-2019.

reported in Tapachula city, Chiapas State and Mexico, respectively. Additionally, correlate of Zika infection in the three serosurveys showed similar risk the whole population (table III).

Discussion

In this work, we described the evolution of Zika seroprevalence in a dengue-endemic municipality in southern Mexico. Due to extensive cross-reactivity between dengue and Zika, it was necessary to conduct an extensive evaluation of the serological tests. Dengue test demonstrated an optimal diagnostic performance. A prevalence above 95% was estimated across the three serosurveys indicating a sustained dengue transmission in Tapachula, Chiapas.

The commercial Zika test showed a specificity greater than 90% in serum samples collected before the Zika outbreak in areas with dengue positivity of less than 25%. Similar results were reported in sera collected from travelers who visited dengue-endemic areas.[14,16,29] However, the specificity dropped to 43.3% in a subsample with 83.4% dengue prevalence, concurrent with another study that reported a 54% specificity in volunteers with previous dengue secondary infections.[17] In our hands, MNT depicted moderate specificity (66.67%). A ZIKV-MNT specificity of 57.1% were reported in French Polynesia,[30] suggesting a poor specificity of ZIKV-MNT in dengue-endemic settings.

Further research is needed to investigate potential immunogenic proteins that can be used as antigens in serological tests. In this work, an in-house ZIKV anti-EDIII IgG ELISA showed the best diagnostic performance. Unfortunately, the availability of the reactive antigens (ZIKV and DENV1 to -4 EDIII) was limited and we were unable to implement the test in our study. We resolved to use the commercial ELISA to detect IgG anti-NS1 recalibrating the cutoff value.

We observed a decline in Zika prevalence over time but a sustained high dengue prevalence. We hypothesize that this could be explained by the specificity maturation of B cells elicited during the infection. The presence of cross-reactive B cells predominates during the first months following the Zika infection, but it wanes over time.[15,31] Our study was concurrent with a lack of ZIKV circulation,[8] that limited re-selection of B cells against ZIKV-NS1 domains would explain the decrease of ZIKV anti-NS1 IgG titers despite a sustained and high dengue prevalence. In fact, increasing DENV anti-NS1 IgG titers were detected probably due to a dengue outbreak that occurred in Tapachula in 2018.[32] These data suggest that immunity to DENV only shapes the breadth and magnitude of antibody response against ZIKV during the first months after the outbreak, producing an overestimated seroprevalence, but years after the ZIKV introduction it is possible to obtain more accurate seroprevalence estimations.

Zika antibody titers did not decrease in participants older than 50 years old despite decreasing was observed in the rest age groups. We have no explanation for this finding, but it is possible that older people may have

Table III
CORRELATES TO ZIKA POSITIVITY. TAPACHULA, CHIAPAS, 2018-2019

Sociodemographic characteristics	*OR*	*95%CI*	*p value*
Age (years)			
0-17	Ref		
18-29	0.66	0.39,1.09	0.105
30-39	0.86	0.29,2.53	0.787
40-49	1.55	0.54,4.44	0.410
50-59	0.98	0.33,2.89	0.977
≥ 60	0.82	0.28,2.45	0.731
Sex			
Male	Ref		
Female	0.66	0.40,1.09	0.105
Education			
Professional or postgraduate	Ref		
Illiteracy	0.63	0.34,1.18	0.152
Elementary school	0.51	0.27,0.95	0.033
Middle school	0.67	0.34,1.36	0.272
High school	0.86	0.40,1.86	0.707
Occupation			
Student	Ref		
Merchant	0.62	0.21,1.82	0.386
Unemployed	1.07	0.21,5.47	0.935
Manual worker	0.46	0.17,1.27	0.134
Households chores	0.90	0.33,2.49	0.844
Professional	1.04	0.32,3.37	0.938
Households incomes ($)			
≥4 501	Ref		
<1 500	1.40	0.81,2.40	0.229
1 501-4 500	0.96	0.58,1.58	0.874
Continuous water supply			
Yes	Ref		
No	1.08	0.74,1.59	0.668
Domestic stored water			
No	Ref		
Yes	1.27	0.87,1.84	0.211

95%CI: 95% confidence intervals; OR: odds rat

more specific anti-DENV mature B cells due to more encounters with DENV and the ZIKV infection elicited a more specific B cell stimulation.[33] In addition, the correlate of Zika infection was similar to all population age groups as we did not find an association in the multinomial model. Similar results were reported in India, suggesting that naïve population had a similar probability to be infected in a first outbreak.[34]

These small-scale serosurveys cannot accurately represent Zika seroprevalence in the state or national populations, but they unveil discrepancies between the extension of Zika outbreak inferred from seroprevalence and the one inferred from official reports. Our estimations in the number of cases at municipality level highlights the underreport of cases during the outbreak that precludes measuring the real extension of the disease. These results do not indicate a need to modify current control measures focused on vector control, although, establishing an accurate denominator in the Zika outbreak will be required in the evaluation of other programs including the widespread vaccination against dengue and Zika.

An important limitation of this study is that the prevalences were estimated in independent serum samples. We assumed that serosurveys could fit the Zika seroprevalence dynamics because the three samples had similar sociodemographic characteristics. Another limitation was the absence of a robust panel of paired serum samples characterized by PCR with detailed historical records of flavivirus infections to extensively evaluate the Zika serological tests. This is a common issue in the Zika seroprevalence studies in dengue-endemic areas, where it is highly unlikely to obtain serum samples from Zika patients without prior dengue infection. The absence of these sera also limited an extensive validation study of dengue serological tests, however, as the study site is a dengue-endemic area, the dengue prevalence overestimation produced by prior Zika immunity would be small.

In conclusion, the Zika outbreak was extensive in Tapachula despite the high dengue immunity in the population. The high Zika and dengue prevalence confirms the underreporting of cases based on the routine flavivirus surveillance system in Mexico. A better performance of the serological test occurs two years after the end of Zika outbreak despite the sustained dengue circulation. Finally, due to the questionable diagnostic performance of the existing serological tests, it is recommended to explore techniques based on type-specific antibody tests, such as the anti-EDIII IgG-based ELISA rather than MNT.

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Declaration of conflict of interests. The authors declare that they have no conflict of interests.

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