The hidden burden of Chikungunya in central Mexico: results of a small-scale serosurvey

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Abstract

Objective. To estimate the seroprevalence of CHKV antibodies and assess correlates of seropositivity at a small geographical scale. **Materials and methods.** A community-based serosurvey of 387 households in Puente de Ixtla, Morelos (central Mexico). Serum IgG antibodies to CHKV were detected by immunoassay. **Results.** From 27 April to 29 May 2016, we interviewed and collected blood samples from 387 individuals at the same number of households. A total of 114 (29.5%) participants were seropositive to CHK, 36 (31.6%) of them reported no symptoms of CHKV infection within 12 months before the survey. **Conclusion.** The estimated seroprevalence to CHKV antibodies was higher than expected by the small number of confirmed cases of CHKV infection reported in Mexico by the National Surveillance System.

Keywords: chikungunya; seroprevalence; epidemiology

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Resumen

Objetivo. Estimar la seroprevalencia de anticuerpos CHKV y evaluar correlatos de seropositividad a pequeña escala geográfica. **Material y métodos.** Encuesta serológica comunitaria en 387 hogares en Puente de Ixtla, Morelos (región central de México). Se detectaron anticuerpos IgG contra CHKV mediante inmunoensayo. **Resultados.** Del 27 de abril al 29 de mayo de 2016 se entrevistó a 387 individuos en el mismo número de hogares y se recolectaron muestras de sangre de los mismos. En total, 114 (29.5%) participantes fueron seropositivos a CHK, 36 (31.6%) de ellos negaron síntomas de infección por CHKV durante los 12 meses previos a la encuesta. **Conclusión.** La seroprevalencia estimada de anticuerpos contra CHKV; fue mayor a la esperada con base en el pequeño número de casos confirmados de infección por CHKV informados en México por el Sistema Nacional de Vigilancia.

Palabras clave: chikungunya; seroprevalencia; epidemiología

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Chikungunya virus (CHKV) has spread globally over the last decade,[1-3] reaching the American continent in November 2013.[4] One year after, sustained transmission of CHKV was identified in Mexico.[5,6] From January 2014 to April 2016, Mexico reported to the Pan American Health Organization (PAHO) an average annual incidence of chikungunya at 4.01 cases per 100 000 inhabitants, which is 22 to 970 times smaller than the average annual incidence of suspect and confirmed chikungunya reported by other countries of America during the same period: Ecuador (89 cases per 100 000 persons), Colombia (380), El Salvador (1 192), or French Guiana (3 859).[7-9]

Although climate, rainfall, urbanization, vector density, housing quality, and social behavior[10,11] may explain varying levels of CHKV transmission across countries, under-detection and under-reporting likely explain Mexico's hidden burden of chikungunya. Most CHKV infections are missed because 20 to 40% of them are asymptomatic, so affected persons do not seek healthcare and are not diagnosed.[12,13] But unlike other countries, Mexico only reports confirmed cases, and its national surveillance guidelines recommend limiting laboratory testing to 5% of the probable cases of acute chikungunya registered at healthcare.[14]

We conducted a small-scale cross-sectional serosurvey to estimate the hidden burden of CHKV infection, and to assess local correlates of seropositivity in Morelos state (central Mexico), a region with low recorded CHKV incidence. Morelos reported its first case of chikungunya in July 2015.

Materials and methods

Study site. The survey was conducted in the town of Puente de Ixtla, Morelos, which spreads over 13.1 square kilometers, encompasses 120 blocks and 8 938 houses, and hosts 34 142 habitants. The local estimated population density was 2 606 habitants per square kilometer.[15] Puente de Ixtla lies at a mean altitude of 900 meters (range, 700 to 2 300) above the sea level. The local climate is mostly warm and semi-arid, with a mean yearly temperature of 24°C (range, 18º C to 28º C) and precipitation of 930 cubic millimeters (range, 800 to 1 200 mm^3).[16,17] The study area included three adjacent census tracts (basic geostatistical areas or AGEB, by its Spanish initials), 0041, 0198 and 0200, that conform a conglomerate of irregular shape between the limits of latitude 18° 36′ 57.3″ North to 18° 61′ 59.29″ South; and longitude 99° 19′ 10.8″ East to 99°31′ 96.62″ West.[18,19]

Population sampling. The study population was selected by probabilistic, multistage, stratified sampling. The AGEBs were selected by convenience using the 2010 National Cartography published by the *Instituto Nacional de Estadística y Geografía* (INEGI). Households within AGEBs were chosen by random sampling proportional to block size. Within each household, a person of the target age (≥ 2 years) was invited to participate. Participants were voluntarily self-selected; those who refuse to participate were replaced by another dweller of the same household. If no person was available or willing to participate at a selected household, the household was replaced by the next one, adjacent to the right, in the same block. A target sample size of 385 participants was calculated to estimate a seroprevalence of at least 10% with 3% precision and 95% confidence level. Assuming a refusal rate of 15%, the target enrollment was 442 persons.

Conduction of the survey. The survey was conducted in three bi-weekly rounds. Study participants answered a face-to-face questionnaire administered by trained personnel. The survey assessed sociodemographic characteristics, housing quality, customary activities, and mobility. It also ascertained self-reported history of dengue and chikungunya at any time, and symptoms compatible with acute and chronic phases of chikungunya within 90 days of the survey. Images of larvae and adult *Aedes spp.* mosquitoes were shown to survey participants for appraising participant's perceived presence of mosquitoes in and around their households. Houses were inspected for open deposits of water, containing mosquito's larvae or eggs.

Blood collection. Interviewees provided a 5-ml blood sample, collected by venipuncture in dry, sterile tubes (Vacutainer No Additive. Becton-Dickinson, Inc.; Franklin Lakes, NJ, USA) immediately after the interview. Participant's fasting was not requested or verified. Blood specimens were stored at 4°C in portable coolers, and remained at this temperature during 7 to 8 hours until they were shipped for testing at the *Centro de Investigación sobre Enfermedades Infecciosas* (CISEI) of the *Instituto Nacional de Salud Pública* (INSP). Samples were centrifuged for 10 minutes at 2 000 rpm to separate the serum, which was immediately stored in 2 ml aliquots in cryovials (Axygen. Corning, Inc.; Union City, California, USA). Sera were stored for 45 to 60 days at -20ºC until testing.

Serological analysis. Sera were thawed at room temperature one hour before testing. Serum IgM and IgG antibodies to CHKV were detected by qualitative

enzyme-linked immunoassay (Euroimmun; Lübeck, Germany) according to manufacturer's recommendations.[20] IgG antibodies were tested in all 387 sera and IgM antibodies were tested only in the 49 sera of participants who reported a clinical diagnosis of chikungunya within 90 days, or a history of compatible symptoms 15 days before the survey. For both, IgG and IgM antibodies, a sample vs. control antibody ratio ≥ 1.1 were regarded positive, and < 0.8 were regarded negative. IgG antibodies to CHKV were interpreted as history of infection at least three months before the survey. Asymptomatic CHKV infection was inferred in participants with a positive test and no history of chikungunya-like symptoms in the year before the survey.

Statistical analysis. Seroprevalence was estimated by the proportion of seropositive individuals in the study population. Correlates of seropositivity were assessed by multivariate logistic regression models using the adjusted odds ratio (aOR) as a measure of association. For all estimates, the 95% confidence interval was the measure of uncertainty. Analyses were performed with STATA, version 14 (Stata Corporation; College Station, Texas). Geographical maps were plotted to show the spatial distribution of seropositive persons, and the number of participants per block who reported *Aedes* mosquitos in or around the house. Plots were made with the ArcGIS Geographic Information System, version 10.3 (Environmental Systems Research Institute (ESRI); Redlands, California).

The study protocol was approved by the ethics, research, and biosafety committees of INSP. Written informed consent was requested from all study participants and the parents of participants aged 2 to 17 years.

Results

From April 27 to May 29, 2016 – during the dry season – 387 persons, living in the same number of households were interviewed and provided a blood sample: 115 in AGEB 0041, 126 in AGEB 0198, and 114 in AGEB 0200 and 32 in an irregular population settlement, adjacent to the AGEB 0041, that was included in the study area.

A total of 290 (74.9%) of the participants were women. Participant's mean age was 42.2 years (range, 2 to 65 years), 283 (73.1%) respondents completed middle school or less, and 181 (46.8%; 95% CI: 41.8, 51.8) reported a monthly household income under 1 500 Mexican pesos. Participant's houses were made of unfinished architecture; 73 (19.6%; 95% CI: 16.0, 23.9) of the houses' roofs were made of asbestos sheet, and 23 (5.9%; 95% CI: 4.0, 8.8) of zinc sheet.

Only 272 (70.2%; 95% CI: 65.5, 74.7) of the participants were continuously supplied with running water, whereas 345 (89.2%; 95% CI: 85.62, 91.9) regularly stored water in open containers. Respondents were mostly sedentary, spending a median of two hours outdoors every day. Overall, 236 (61.0%; 95% CI: 56.0, 65.8) respondents had identified adult mosquitoes in or around their houses (table I).

Forty-seven (12.2%; 9.2, 15.8) interviewees reported a history of dengue in themselves, 44 (11.3%; 95% CI: 8.6, 15.0) in a household member; and 83 (21.5%; 95% CI: 17.6, 25.8) reported history of chikungunya, within a year of the survey. Interviewees in 23 (20%) of the 114 blocks sampled reported *Aedes spp* (figure 1) mosquitoes in or around the house, and mosquito breeding sites in 54 (47%) blocks (figure 2). Finally, a ravine runs for approximately 840 meters across the study area; all four blocks immediately adjacent to the ravine had at least one seropositive person.

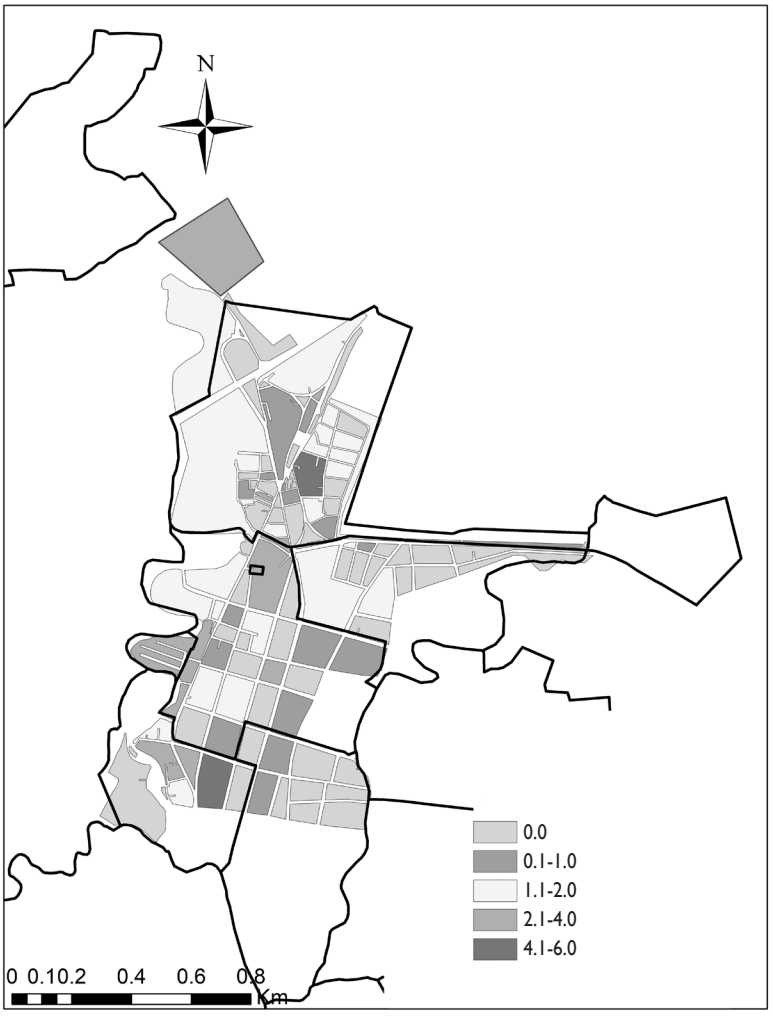
A total of 114 (29.5%; 95% CI: 25.1, 34.2) of the 387 participants were seropositive to IgG antibodies to CHKV. The seroprevalence of CHKV was larger in those with a reported history of chikungunya within a year of the survey (78.3%; 95% CI: 68.1, 80.0) than those without it (16.1%; 95% CI: 12.4, 20.7), and in respondents who reported a history of dengue (42.9%; 95% CI: 33.0, 53.3) compared to those without it (25.3%; 95% CI: 20.7, 30.6), and in participants who had observed adult mosquitoes in or around their houses (33.9%; 95% CI: 28.1, 40.2) than in those who had not observed mosquitoes (22.5; 95% CI: 16.5, 29.9). All 49 sera tested for IgM antibodies were negative.

We fitted a multivariate logistic regression model. According to the model, four correlates were independently associated with seropositivity to CHKV antibodies: self-reported history of chikungunya within a year (aOR= 23.4; 95% CI: 12.0, 45.4), self-reported history of dengue in the participant or in a household member (2.6; 95% CI: 1.4, 4.9), house roof made of asbestos sheet (2.8; 95% CI: 1.4, 5.3), and *Aedes spp*. mosquitoes observed in or around the house (1.9; 95% CI: 1.1, 3.5). A monthly household income above 1 500 *pesos* appears to be inversely associated with CHKV seropositivity (0.6; 95% CI: 0.3, 1.0) (table II).

Table I

Characteristics of the 387 study participants. Chikungunya serosurvey in Puente de Ixtla, Morelos, México. April 27 to May 29, 2016

Characteristic	*Seronegative (n = 273)*			*Seropositive (n = 114)*			*Total (n = 387)*		
	Mean, 95% confidence interval								
Age, years	42.29		(39.99-44.59)	42.00		(38.47-45.53)	42.21		(40.29-44.13)
	N, %, 95% confidence interval								
Female	200	73.26	(67.67-78.20)	90	78.94	(70.43-85.51)	290	74.94	(70.35-79.02)
Formal education									
University	37	13.55	(9.96-18.17)	7	6.14	(2.94-12.38)	44	11.37	(8.56-14.95)
High school	44	16.12	(12.20-20.99)	16	14.04	(8.75-21.76)	60	15.50	(12.22-19.48)
Middle school	98	35.90	(30.40-41.79)	49	42.98	(34.16-52.27)	147	37.98	(33.26-42.95)
Elementary	67	24.54	(19.78-30.02)	31	27.19	(19.77-36.14)	98	25.32	(21.22-29.92)
None	24	8.79	(5.95-12.80)	11	9.65	(5.40-16.65)	35	9.04	(6.56-12.35)
Other	3	1.09	(0.35-3.37)	0	-	-	3	0.78	(0.25-2.39)
Occupation of the interviewee									
Professional	20	7.33	(4.76-11.10)	6	5.26	(2.37-11.28)	26	6.72	(4.61-9.70)
Manual worker	16	5.86	(3.61-9.37)	8	7.02	(3.53-13.47)	24	6.20	(4.18-9.10)
Merchant	20	7.33	(4.76-11.10)	10	8.77	(4.76-15.60)	30	7.75	(5.46-10.89)
Unemployed	164	60.07	(54.12-65.74)	76	66.67	(57.46-74.75)	240	62.02	(57.05-66.74)
Student	23	8.43	(5.65-12,38)	10	8.77	(4.76-15.60)	33	8.53	(6.12-11.77)
Other	28	10.26	(7.16-14.48)	4	3.51	(1.31-9.04)	32	8.27	(5.90-11.48)
Does not apply	2	0.73	(0.18-2.90)	0	-	-		0.52	(0.13-2.05)
Monthly household income, pesos									
<1 500	118	43.22	(37.44-49.20)	63	55.26	(45.99-64.18)	181	46.77	(41.82-51.78)
1 500-4 499	101	37.00	(31.45-42.91)	38	33.33	(25.25-42.54)	139	35.91	(31.27-40.85)
4 500-7 499	45	16.48	(12.52-21.39)	11	9.65	(5.40-16.65)	56	14.47	(11.29-18.36)
7 500-9 999	8	2.93	(1.47-5.77)	2	1.75	(0.44-6.81)	10	2.58	(1.39-4.74)
10 000+	1	0.37	(0.05-2.58)	0	-	-	1	0.26	(0.04-1.83)
House roof material									
Concrete	210	76.92	(71.53-81.56)	72	63.16	(53.88-71.55)	282	72.86	(68.20-77.08)
Asbestos sheet	43	15.75	(11.88-20.59)	33	28.94	(21.32-38.00)	76	19.64	(15.96-23.92)
Zinc sheet	15	5.50	(3.33-8.93)	8	7.02	(3.53-13.47)	23	5.94	(4.00-8.80)
Other	5	1.83	(0.76-4.34)	1	0.88	(0.12-6.04)	6	1.55	(0.70-3.42)
Continuous supply to water	196	71.79	(66.14-76.84)	76	66.67	(57.46-74.75)	272	70.28	(65.52-74.65)
Store water in open deposits	242	88.65	(84.28-91.91)	243	90.35	(83.35-94.60)	345	89.15	(85.62-91.89)
Average time spent outdoors, hours									
< 1	45	16.48	(12.52-21.39)	23	20.18	(13.75-21.39)	68	17.57	(14.08-21.70)
1-2	91	33.33	(27.97-39.17)	42	36.84	(28.45-46.12)	133	34.36	(29.78-39.26)
3-7	63	23.08	(18.44-28.48)	23	20.17	(13.75-26.61)	86	22.22	(29.78-39.26)
8-10	55	20.25	(15.78-25.35)	18	15.79	(10.15-23.74)	73	18.86	(15.25-23.09)
10 +	19	7.00	(4.47-10.68)	8	7.02	(3.53-13.47)	27	6.98	(4.82-10.00)
Aedes spp. mosquitoes observed in or around the house									
Larvae	37	13.55	(9.96-18.17)	27	23.68	(16.73-32.41)	64	16.54	(13.15-20.60)
Adult forms	156	57.14	(51.17-62.92)	80	70.18	(61.10-77.90)	236	60.98	(56.01-65.74)
Dengue in a household member	30	10.99	(7.78-15.51)	14	12.28	(7.38-19.74)	44	11.34	(8.56-14.95)
Self-reported history of dengue	22	8.06	(5.35-11.86)	25	21.93	(15.23-30.52)	47	12.15	(9.24-15.81)
Self-reported history of chikungunya	18	6.59	(4.18-10.24)	65	57.02	(47.73-65.84)	83	21.45	(17.63-25.84)



Fuente del mapa: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User community

FIGURA 1. NUMBER OF CHKV POSITIVE INDIVIDUALS PER BLOCK. PUENTE DE IXTLA, MORELOS, MÉXICO, APRIL 27 TO MAY 29

Discussion

To our knowledge, this is the first published report of CHKV serosurvey in Mexico. Our results suggest that in the town of Puente Ixtla, Morelos, 29.5% of the population aged between 2 years and 65 years were infected by CHKV in the first 18 to 20 months of its emergence in Mexico.[21]

We found that self-reported history of chikungunya and dengue were strong predictors of seropositivity to CHKV. Up to 16% of the study participants who denied history of disease, were seropositive to CHKV. This is consistent with the range of 5 to 40% of asymptomatic CHKV infections previously described by other authors.[22-24] Although chikungunya, dengue, and zika are similar diseases that can be misclassified on clinical grounds, the severity of symptoms facilitates recognition of chikungunya and, therefore, the proportion of unapparent infections is typically lower than that in dengue (61% to 74%) or zika (80%). [25–27]

Almost two fifths of the seropositive participants (39.5%) in our survey reported persistent pain, limited mobility, or lasting arthralgia. Long-lasting CHKV disease is a matter of public health concern as it may

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Figura 2. Number of participants per block that report presence of vector Ae. aegypt in its larval and adult form. Puente de Ixtla, Morelos, México April 27 to May 29

Table II

Correlates of seropositivity to chikungunya antibodies in 387 study participants of the chikungunya serosurvey in Puente de Ixtla, Morelos, México. April 27 to May 29, 2016

Characteristic	*Adjusted odds ratio**	*95% confidence interval*
Self-reported history of chikungunya	23.36	(12.01-45.44)
Self-reported history of dengue in the participant or a household member	2.60	(1.40-4.86)
Asbestos sheet roof	2.75	(1.43-5.32)
Aedes spp. mosquitoes observed in or around the house	1.93	(1.06-3.53)
Monthly household income above 1 500 pesos	0.58	(0.33-1.03)
Continuous supply of water	0.65	(0.35-1.19)

* Estimates are adjusted by all other characteristics listed in the table

lead to considerable impairment of their quality of life, reduced productivity, and economic loss in the families and the community as has been described in other studies.[23,28]

Other authors have discussed poverty and low levels of education as predictors of the risk of arbovirus infections.[28,29] We explored socioeconomic and demographic characteristics as potential correlates of CHKV seroprevalence. House quality (asbestos roof materials) was strongly associated to CHKV seropositivity. Other variables such as low household income, level of formal education, and occupation were apparently associated with seropositivity, but our results were statistically inconclusive likely because our relatively small sample size, and the narrow variability of socioeconomic characteristics in the study population.

This small-scale serosurvey cannot accurately represent CHKV seroprevalence in the state or national populations, but it shows a remarkable gap between the burden of chikungunya inferred from seroprevalence and that inferred from surveillance reports of confirmed cases. Seroprevalence and cumulative incidence of infection certainly convey different information but, under certain assumptions, the rate or seroconversion in infected individuals may allow linking these two data streams. Assuming that infection elicits long-lasting immunity and considering that a large proportion of CHKV infections are asymptomatic, seroprevalence is expected to be larger than incidence. Our estimates of seroprevalence sharply contrast with the small number of cumulative confirmed CHKV infections reported in Puente de Ixtla (19 cases), Morelos (400 cases) and Mexico (11 865 cases) in early April 2016.* The estimated incidence of 29.5% would translate into 768 cases in Puente de Ixtla. If we further assume similar risk of infection across the state and the country, we would expect 560 862 cumulative CHKV cases in Morelos and 37 573 402 in Mexico.

Finally, Mexican surveillance guidelines recommend testing for CHKV infection only 5% of the symptomatic cases who seek healthcare but the proportion that is truly tested remains unknown. Therefore, caution should be exercised when interpreting epidemiological reports that regard confirmed cases as the only indicator of the burden chikungunya, while enclosing critical methodological information that would allow interpreting surveillance data in the right context.

* Vigilancia Epidemiológica, Enfermedades Transmitidas por Vector. Chikungunya 2015-2016, Jursidicción Sanitaria No. II, Jojutla, Morelos. 2016. Datos no publicados.

Conclusions

We estimated the seroprevalence of IgG antibodies to CHKV at 29.5%, in a small-scale serosurvey in central Mexico. This suggests extensive transmission of CHKV during the epidemic phase in 2015 and contrasts with the notion of low transmission conveyed by surveillance reports. We claim that greater transparency and integrated approaches to assess epidemics may improve the credibility and usefulness of surveillance in Mexico.

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

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