Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

has been the fact that Iran's Ministry of Health and Medical Education (MoHME) stopped releasing province-level data on the number of confirmed COVID-19 cases from March 22, 2020, onward. Furthermore, provincial data on the number of confirmed COVID-19-related deaths were never released. Instead, MoHME reports the percentage change in the number of cases with respect to previous days as an indicator of the state of the epidemic in each province and colour-codes them from blue (low incidence) to yellow (medium incidence), orange (high incidence), and red (very high incidence).

Despite the significant implications of understanding the Iranian epidemic for the country and the Eastern Mediterranean region as a whole, research investigations have largely been hindered due to the lack of epidemiological data on the number of cases and deaths, age-stratified and sex-stratified data, both at the national and province level, and seroepidemiological analysis.[1] The study by Hossein Poustchi and colleagues,[2] sponsored by MoHME and carried out by the then Deputy Minister of Research and Technology of the Ministry of Health Reza Malekzadeh and his team, to measure SARS-CoV-2 antibody seroprevalence in the general population across 18 cities of Iran was the first systematic investigation into the geographical spread of COVID-19 across the country nearly a year after the first two cases were reported in Qom on Feb 19, 2020. Their analysis showed greatly varied levels of exposure in different cities, with some reaching very high levels (>50% in Qom and Rasht) by late April to early June.

Before the study by Poustchi and colleagues, we did a similar province-level analysis using seasonal all-cause mortality data to estimate the excess mortality in all 31 provinces of Iran from winter to summer, 2020.[3] Our findings corroborate the results by Poustchi and colleagues (appendix p 1), with an overall significant correlation (R^2=0·67 and p<0·001; appendix p 2). Our results further suggest that most provinces would continue to have a two to four times increase in exposure until the end of summer (Sept 21, 2020), with Qom and Golestan reaching approximately 57% (95% CI 44–69) population-level exposure.[3]

In the absence of more recent serology or province-level data, our estimates provide the most recent indicator of prevalence. This comparison is of immediate epidemiological importance as it highlights areas with the largest epidemic growth, which require the most immediate interventions. The continued availability of province-level data would be of paramount public health importance in a country that is facing such a heavy toll from COVID-19.

We declare no competing interests.

*Mahan Ghafari, Alireza Kadivar, *Aris Katzourakis
mahan.ghafari@zoo.ox.ac.uk; aris.katzourakis@zoo.ox.ac.uk

Department of Zoology, University of Oxford, Oxford, UK (MG, ArK); and Center for Statistics and Operation Research, Statsminute, Tehran, Iran (AlK)

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4 Shakiba M, Nazemipour M, Salari A, et al. Seroprevalence of SARS-CoV-2 in Guilan province, Iran, April 2020. *Emerg Infect Dis* 2020; published online Dec 21. https://doi.org/10.3201/eid2702.201960.

Hossein Poustchi and colleagues[1] reported SARS-CoV-2 antibody seroprevalence in the general population and high-risk occupational groups across 18 cities in Iran. However, there are several major concerns regarding their study design, analysis, and results.

First, although appendix 2 of the Article mentions cities as clusters, it is unclear how these clusters were selected. The inverse of the selection probability of individuals, which is a function of probability of cluster selection and unknown here, should be used as weights in the analyses.[2]

Second, the sample size calculation has errors. In the design effect formula, n is calculated as $\sum m^2 / \sum m = 1180{\cdot}2$ (where m is the cluster size)[3] but not the number of clusters (n=18), as mentioned in appendix 2. Also, the intracluster correlation (δ) of 0·05 is too high for large clusters (such as those encountered in the study by Poustchi and colleagues), without any supporting references. Furthermore, it is unclear whether the seroprevalence (p) of 0·15 refers to the general population or high-risk groups, and again no references are given on the reported value.

Third, the bootstrap procedure described in appendix 2 mimics simple random sampling and does not consider clustering in the design, leading to too narrow confidence intervals (CIs). In fact, the appropriate bootstrapping procedure for cluster designs would draw the cluster units rather than individual units with replacement. Alternatively, one can use cluster-robust standard errors.[4] The CIs are also narrow due to uncertainties in the sensitivity and specificity estimates. A Monte-Carlo bias analysis from an appropriate probability distribution of sensitivity and specificity can be used for overcoming this problem.[5]

Fourth, a seroprevalence of 72·6% for Rasht city seems to be an overestimate and inconsistent with

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See **Online** for appendix

the results of other studies, which reported estimates of about 23·7% for Rasht and 27·5% for Guilan province in April and mid-June, respectively.[5,6] This difference cannot be attributed to the different design and analysis of those studies. Moreover, a SARS-CoV-2 seropositive status seems to be durable (at least up to 8 months after infection)[7] and can probably protect people from reinfection.[8] The alarming (red) status of Rasht during the previous months[9] is not consistent with Poustchi and colleagues' estimated seroprevalence, which is higher than the presumed threshold of COVID-19 herd immunity (50–67%).[10]

Finally, as seroepidemiological studies can affect decisions related to immunisation programmes and pandemic control measures, we believe that the results of Poustchi and colleagues' study should be more carefully interpreted, and we hope for studies with more robust design and analysis.

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*Maryam Nazemipour, Maryam Shakiba, *Mohammad Ali Mansournia*
mansournia_ma@yahoo.com

Psychosocial Health Research Institute, Iran University of Medical Sciences, Tehran, Iran (MN); Cardiovascular Diseases Research Center and School of Health, Guilan University of Medical Sciences, Rasht, Iran (MS); Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran (MAM)

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Authors' reply

We thank Mahan Ghafari and colleagues and Maryam Nazemipour and colleagues for their comments on our study reporting the seroprevalence of SARS-CoV-2 antibodies in 18 cities of Iran.[1] Our findings of considerable variation in seroprevalence rates by city and high exposure levels in Rasht and Qom are supported by Ghafari and colleagues, as they observed similar trends in province-level excess mortality rates in the same regions.[2] These findings are consistent with a high incidence of COVID-19 in a few cities of northern (eg, Rasht in Gilan province) and central (eg, Qom in Qom province) provinces of Iran (red colour-coded regions), as reported by the Ministry of Health and Medical Education (MoHME) early in the pandemic (April–June, 2020).[2] Furthermore, in the seventh report of MoHME, summarising the results of scattered seroepidemiological studies in Iran, among blood donors the prevalence of anti-SARS-CoV-2 antibodies in Gilan province was 55·0% (95% CI 38·0–71·0),[3] with CIs that overlap with the CIs of our estimate in Rasht (72·6%, 95% CI 53·9–92·8).

Conversely, Nazemipour and colleagues stated that our seroprevalence for Rasht was overestimated.[2,4] Their argument was mainly based on the reported seroprevalence of 23·7% in Gilan province in a study by Shakiba and colleagues[4]—a study with several limitations, including a low participant response rate (31·0%) and inadequate information on test characteristics. Although the test-adjusted estimate for Rasht in our study was high, its crude estimate was 58·6%, representing the effect of test characteristics on assessed prevalence (ie, higher prevalence and lower test sensitivity would result in a higher adjusted estimate). The observed variation in adjusted seroprevalence estimates between different studies is partly related to differences in test characteristics. Hence, in addition to test sensitivity and specificity, providing their CIs could indicate the expected variation in a prevalence estimate. In Shakiba and colleagues' study, the CIs for VivaDiag test performance were not assessed.[4] Therefore, the concern raised by Nazemipour and colleagues that the seroprevalence for Rasht was overestimated and inconsistent with other studies is neither supported by our data nor by other studies.

Since the incidence of COVID-19 in Rasht city remained high during the past few months, Nazemipour and colleagues also stated that our reported 72·6% seroprevalence estimate for Rasht did not follow the presumed threshold for herd immunity. We disagree with this statement as the current evidence on herd immunity and its association with antibody status is still lacking, and a high level of exposure (ie, >50%) is not a sufficient indicator for herd immunity against COVID-19.[5] This assumption requires further investigation and could adversely affect the current applied health regulations and vaccination programmes in the country.

Finally, Nazemipour and colleagues highlighted some points with respect to our analytical approach,