Trained immunity' from Mycobacterium spp. (environmental or BCG) exposure predicts protection from Coronavirus disease 2019 (COVID-19)

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ABSTRACT:
Endeavors to identify protective variables that could be potentially responsible for reduced COVID-19 impact on certain populations have remained a priority. Multiple attempts have been made to attribute the reduced COVID-19 impact on populations to their bacillus Calmette–Guérin (BCG) vaccination coverage ignoring the fact that the effect of childhood BCG vaccination wanes within the first 5 years of life while most of the COVID-19 cases as well as deaths have been observed in adults especially the aged with comorbidities. Since the supposed desired protection being investigated could come from heterologous ‘trained immunity’ conferred by exposure to Mycobacterium spp. (i.e., environmental and BCG), it is argued that the estimates of the prevalence of ‘trained immunity’ of populations currently available as latent tuberculosis infection (LTBI) of populations would be a better variable to evaluate such assertions. Indeed, when we analyze the European populations (twenty-four) as well as erstwhile East and West Germany populations completely disregarding their BCG vaccination coverage, the populations with higher trained immunity prevalence consistently display reduced COVID-19 impact as compared to their lower trained immunity prevalence neighbors. The incidences, mortality, and interim case fatality rates (i-CFR) of COVID-19 are found negatively correlated with the trained immunity of populations that have comparable underlying confounders not the BCG coverage per se. It is submitted that to decisively arrive at dependable conclusions about the potential protective benefit that can be gained from BCG vaccination in COVID-19, the ongoing/planned randomized controlled trials should consciously consider including measures of trained immunity as - a) all individuals immunized do not respond equally, b) small study groups of higher background trained immunity could fail to indicate any protective effect.

Key Words: COVID-19, Trained Immunity, bacillus Calmette–Guérin, BCG, Tuberculin Sensitivity Test (TST), pandemic, SARS-CoV-2

NOTE: This preprint reports new research that has not been certified by peer review and should not be used to guide clinical practice.
INTRODUCTION

There have been efforts to understand and explain the differential impact of COVID-19 on populations in pursuance of identifying protective variables that could predict the impact or be applied for intervention. Recent studies in PNAS and Science Advances (1,2) had endeavored to explain/model the differential effect on populations based on ‘trained immunity’ correlates of countries’ as per bacillus Calmette–Guérin (BCG) vaccination rates after meticulous correction/fitting of the data for supposed major confounders like age, population density, development status, BCG coverage/implementation using the infections and mortality data from an early stage of pandemic (till April 22). More recently, other communications in PNAS (3,4) have failed to find support for the association previously observed between BCG and the impact of COVID-19 on populations when using updated data set.

The extrapolation of associative observations made previously (1,2) linking BCG vaccination coverage to reduced COVID-19 impact on populations was expected to disappear (3-5) as the ‘trained immunity’ conferred by childhood BCG vaccination usually wanes in <5 years (5-7). Hence, the premise of protective ‘trained immunity’ from BCG vaccination given in childhood or to children in a population is not supposed to decrease the severity of infection or supposedly provide any protection in currently aged as the BCG conferred ‘trained immunity’ correlates would have waned away long ago (5-7). The use of early-stage pandemic data (1,2) when the populations were not evenly exposed along with displayed associations’ inherent disconnect with the mechanism proposed behind the observed protective correlation make such assertions untenable.

We reason, the dependability on the correlative associations as well as conclusions presented in refs. (1-4) would tremendously improve on considerations: a) direct measure of prevailing supposed protective ‘trained immunity’ correlate (TIC) as a result of populations exposure to Mycobacterium spp. or BCG vaccination (5,6,7), i.e., Tuberculin positivity [TIC of BCG given at birth wanes within <5 years (6), so chances of heterologous protection (8) of elderly from childhood vaccination are remote]; b) analysis of countries at a similar stage of the pandemic; c) underlying confounders including potential contributory variables (e.g., Vitamin D, Zinc) (9,10); d) the correlations observed, at any time, to be the total sum of the effects from protective variable and preventive/curative measures in place (e.g., social distancing norms and adherence, medical infrastructure/support).

The European populations with quite dissimilar BCG coverage (including no vaccination) that have experienced differential COVID-19 impact [11] offer an excellent opportunity to evaluate the alternative hypothesis that the ‘trained immunity from Mycobacterium spp. exposure (BCG or environmental NOT
the childhood vaccination coverage per se) could be protective in COVID-19. It would be theoretically better equipped to predict the flattened curve if any such association exists, that may have cause and effect relationship. The analysis of TIC and COVID-19 data from 24 socially similar European countries support a potential protective role for the prevalent TIC of populations on COVID-19 infections and mortality.

**MATERIAL AND METHODS:**

The COVID-19 incidence and mortality data for the European countries (Supp. Table 1) was obtained from Worldometer (11; https://www.worldometers.info/coronavirus/ [Accessed on 27 August]) and that of East and West Germany states from https://www.citypopulation.de/en/germany/covid/ [Accessed on 10 October 2020] and previously published estimates (7). The latent tuberculosis infection (LTBI) (12) prevalence estimates (i.e., ‘trained immunity’ correlate) were from reference (13). All statistical estimations and correlation analysis of the COVID-19 incidence and mortality with TIC or LTBI prevalence of populations (Average, Standard deviation (STDEV), Standard Error (Std. Err.), F-value, Correlation/ Pearson coefficient (r/R), regression, etc) were performed using Microsoft Excel 2019. The p-values <0.05 were considered significant unless explicitly stated otherwise. The methodology employed has been essentially the same as described previously (5,7,9,10).

**RESULTS & DISCUSSION:**

Analysis of the updated COVID-19 data (till 28 August, Sup. Table 1, ref. 11) from 24 European countries with similar confounders (ref.1 and additional), stage of the pandemic, without any exclusions (applied in ref. 1. and 3.) consistently displayed protective/negative correlation with the direct measure of desired heterologous TIC of populations [i.e., tuberculin positivity without active tuberculosis disease; referred by WHO as Latent tuberculosis infection (LTBI) for the management purposes (12-13)]. Higher LTBI populations consistently displayed lower COVID-19 incidence/mortality per million and interim case fatality rates (i-CFR) (Fig. 1). The correlations observed shown here are supposed to decline further before the disappearance of COVID-19 due to progressive loss of synchronicity of infections/pandemic-phases (Sup. Fig. 2), lower prevalence of the protective variable (6.06 - 15.95%) (13), the differential response of the study population, etc. not necessarily the supposed absence of correlation (3).
Similarly, East and the West Germany States displayed significant negative covariation of cases, mortality, and i-CFR rates with estimated TIC of populations (22.5% and 9.2% LTBI, respectively) without requiring correction factors (10 April – 28 August; Fig. 2). Expectedly, the differential response gap seen for East and West Germany is showing signs of closing as expected for populations slowly reaching towards stable equilibrium with underlying confounders (Fig. 2C).

In conclusion, we believe the incidences, mortality, and i-CFR of COVID-19 would negatively correlate with the trained immunity of populations that have comparable underlying confounders, not the BCG coverage \textit{per se}. To decisively arrive at dependable conclusions about the potential protective benefit of BCG in COVID-19, the ongoing/planned randomized controlled trials should consciously consider including measures of TIC \cite{14,15} as - a) all individuals immunized do not respond equally, b) small study groups of higher background trained immunity could fail to indicate any protective effect. Currently under development COVID-19 vaccines still have a long way to go and be available in sufficient supply to cover the whole global population at the same time to confer the much-needed ‘herd immunity’ whereas BCG is readily available and may give us respite. Any potential protective effect displayed by BCG vaccination in the ongoing trials, especially in aged and persons with comorbidities who are currently accounting for more than 90% deaths, could help provide hope in the current scenario.

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\textbf{Author contribution:} SS designed the research, analyzed the data, and wrote the paper; RK contributed to analysis and drafting.

\textbf{REFERENCES:}

1. Escobarla LE, Molina-Cruzb A, Barillas-Mury C. BCG vaccine protection from severe coronavirus disease 2019 (COVID-19). PNAS 2020; 117: 17720-17726; www.pnas.org/cgi/doi/10.1073/pnas.2008410117

2. Berg MK, Yu Q, Salvador CE, Melani I, Kitayama S. Mandated Bacillus Calmette-Guérin (BCG) vaccination predicts flattened curves for the spread of COVID-19. Sci Adv. 6, eabc1463 (2020).
3. Arlehamna CSL, Settea A, Peters B. Lack of evidence for BCG vaccine protection from severe COVID-19. PNAS 2020; 117: 25203–25204; www.pnas.org/cgi/doi/10.1073/pnas.2016733117

4. Patella V, Delfino G, Bruzzese D, Giuliano A, Sanduzzi A. The bacillus Calmette–Guérin vaccination allows the innate immune system to provide protection from severe COVID-19 infection. PNAS 2020, 117 (41) 25205-25206; DOI: 10.1073/pnas.2015234117

5. Singh S. BCG Vaccines May Not Reduce Covid-19 Mortality Rates. medRxiv 2020.04.11.20062232. https://doi.org/10.1101/2020.04.11.20062232

6. Menzies D. Interpretation of repeated tuberculin tests. Boosting, conversion, and reversion. Am J Respir Crit Care Med. 1999 Jan; 159(1):15-21

7. Singh S, Maurya RP, Singh RK (2020) “Trained immunity” from Mycobacterium spp. exposure or BCG vaccination and COVID-19 outcomes. PLoS Pathog 16(10): e1008969. https://doi.org/10.1371/journal.ppat.1008969

8. O’Neill LAJ, Netea MG. BCG-induced trained immunity: can it offer protection against COVID-19?. Nat Rev Immunol 2020; 20: 335–337. https://doi.org/10.1038/s41577-020-0337-y

9. Singh S, Kaur R, Singh RK. Revisiting the role of vitamin D levels in the prevention of COVID-19 infection and mortality in European countries post infections peak. Aging Clin Exp Res. 2020;32(8):1609-1612. doi:10.1007/s40520-020-01619-8

10. Singh S. Covariation of Zinc Deficiency with COVID19 Infections and Mortality in European Countries: Is Zinc Deficiency a Risk Factor for COVID-19? J Sci Res. 2020;64(2):153–157.

11. Worldometer. COVID-19 Coronavirus Pandemic. https://www.worldometers.info/coronavirus/ [Accessed on 27 August]

12. WHO. Latent tuberculosis infection: updated and consolidated guidelines for programmatic management. 2018. ISBN 978-92-4-155023-9. [Available at https://apps.who.int/iris/bitstream/handle/10665/260233/9789241550239eng.pdf;jsessionid=24D3F1097E3884502A9C1A1698348491?sequence=1]

13. Global Burden of Disease Collaborative Network. Global Burden of Disease Study 2017 (GBD 2017) Results. Seattle, United States: Institute for Health Metrics and Evaluation (IHME), 2018. [Available from http://ghdx.healthdata.org/gbd-results-tool; search term combination “Prevalence - Latent Tuberculosis Infection - Sex: Both - Age: All Ages (Percent)”]

14. BCG vaccination and COVID-19 trials (19) listed at https://clinicaltrials.gov/ct2/results?cond=COVID-19&term=BCG&ctry=&state=&city=&dist=&Search=Search

15. Hamiel U, Kozer E, Youngster I. SARS-CoV-2 Rates in BCG-Vaccinated and Unvaccinated Young Adults. JAMA. 2020; 323(22): 2340-2341. doi:10.1001/jama.2020.818
**Fig. 1.** The COVID-19 cases, deaths and CFR consistently remained lower in European populations with higher trained immunity (TI) correlate (>10% LTBI) post-peak-of infections than countries with the lower TI-correlate (<10% LTBI). The TI-correlate indicated a significant consistently negative association with COVID-19 infections [Pearson correlation $r(24)=-0.79$ to $-0.57$, p-value<0.005] and mortality [$r(24)=-0.63$ to $-0.45$, p-value<0.05)] for the whole time period (12 March to 26 August 2020). See Sup. Fig 1 in conjunction with Sup. Fig. 2 for detailed correlation analysis and its variation with wave of infections across 24 countries. Refer to Sup. Table 1 for updated COVID-19 cases and deaths data for the 24 countries with supposedly similar confounders and at similar stage of pandemic included in the study. (A) The i-CFR [(deaths/cases)*100] for Low LTBI countries had remained higher than that of high LTBI countries post infections peak. (B) Low LTBI countries have had relatively higher infections/million population (1.63 fold on 26 August) and consistently higher i-CFR (~30% on 26 August).
Fig 2. East Germany states with higher trained immunity correlate (%LTBI) as compared to West Germany states (22.5% vs 9.2%) consistently reported lower COVID-19 cases (A), deaths (B) and i-CFR (C) during study period (10 April to 28 August). Refer to Sup. Table 2 for COVID-19 cases and deaths and Sup. Table 3 for i-CFR estimates. E. Germany consistently had 20-30% lower CFR as compared to W. Germany states. The inclusion of Berlin in East Germany region, and that of Hamburg and Bremen in West Germany region decreased the closing trend of the i-CFR with passage of time (compare covariation of Red and Green trend lines with Orange and Light green in (C) possibly indicative of more LTBI positives in Berlin than in Hamburg and Bremen. In future, as the pandemic progresses the gap between E. and W. Germany states is expected to close, partially resulting from decrease in the vulnerable population and the concomitant increase in populations overall ‘trained immunity’ as a result of infections.
Sup. Fig.1. The COVID-19 cases (A) and Deaths (B) in European countries with similar confounders and stage of pandemic consistently remained negatively and significantly correlated with Trained immunity prevalence (est. %LTBI) starting from March 12 to August 26. See Supp. Fig 2 for correlation analysis for the period starting from 12 March to 26 August 2020 covering the duration upto April 22 of ref.1 and August 1 reference point of ref. 2.
Sup. Fig 2. The correlation between underlying prevailing trained immunity correlate (%LTBI) of European populations with COVID-19 cases per million (A) and deaths per million (B) and its dependence on phase of pandemic. The observed correlation (see bottom correlation analysis table) consistently remained negative for the period. The notification rates for countries with >10% LTBI is indicated by broken lines. Correlation remained high with the synchronicity of 1st peak of infections (see corresponding notification rates graph above for the data date indicated in the table below) and been on decline since then partially resulting from the loss of synchronicity, populations response, acquired immunity and understandably and importantly the changing reporting and management practices. Refer to Sup. Fig. 3 from European CDC that more accurately reflects the waves of infections/deaths from starting not explicitly observable in the figure one presented here due to coarse methodology employed. The response of populations had been more stringent and uniform for 1st wave of infections. Note: The highlighted 12-May and 26-May values could reflect the assumed total sum of actual maximum achievable correlation for potential ‘trained immunity’ along with current confounders and the stringent measures put in place by the countries to reduce the spread of COVID-19 – NOT necessarily due to only the prevailing trained immunity of the populations as a result of BCG coverage or implementation alone as assumed (ref.1 and 2.). Even if there is a cause and effect relationship, the expected protective covariation (correlation) would expectedly further go down for reasons mentioned above. It may soon reach 0.2-0.4 for the study populations primarily due to increasingly heterogenous (loosened) response combined with actual low trained immunity prevalence).
### Table 1. COVID-19 data [Cases per million (CpM) and deaths per million DpM] of European countries

| Countries | CpM 12 Mar | DpM 12 Mar | CpM 26 Mar | DpM 26 Mar | CpM 12 Apr | DpM 12 Apr | CpM 26 Apr | DpM 26 Apr | CpM 12 May | DpM 12 May | CpM 26 May | DpM 26 May | CpM 12 Jun | DpM 12 Jun | CpM 26 Jun | DpM 26 Jun | CpM 12 Jul | DpM 12 Jul | CpM 26 Jul | DpM 26 Jul | CpM 12 Aug | DpM 12 Aug | CpM 26 Aug | DpM 26 Aug | CpM 12 Sep | DpM 12 Sep | CpM 26 Sep | DpM 26 Sep |
|-----------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| Denmark   | 116.30     | 0.00       | 323.87     | 7.07       | 1065.31    | 47.11       | 1479.60    | 72.82       | 1827.45    | 90.93      | 1971.87    | 97.14       | 2072.12     | 104.22      | 2388.04    | 105.08      | 2388.04    | 105.08      | 2388.04    | 105.08      | 2388.04    | 105.08      | 2388.04    | 105.08      |
| France    | 4.44       | 0.00       | 3.96       | 4.46       | 1560.71    | 220.22      | 1907.82    | 349.69      | 2147.53    | 412.99     | 2229.12    | 436.54      | 2393.48    | 449.47      | 2495.31     | 455.66      | 2636.48    | 459.23      | 2793.92    | 462.50      | 3165.46    | 468.12      | 3838.66    | 476.67      |
| Germany   | 3.75       | 0.07       | 92.65      | 1.85       | 1002.15    | 3.19       | 1525.22    | 6.08        | 2058.81    | 9.21       | 2162.41    | 10.38       | 2322.78    | 10.73       | 2391.04    | 10.96       | 2469.27    | 10.96       | 2469.27    | 10.96       | 2469.27    | 10.96       | 2469.27    | 10.96       |
| UK        | 8.19       | 0.00       | 1.28       | 0.95       | 2791.10    | 0.03       | 479.92     | 1.85        | 513.92     | 4.23       | 997.82     | 4.96        | 1236.02    | 4.96        | 1302.65    | 4.96        | 1388.92    | 4.96        | 1484.07    | 4.96        | 1587.13    | 4.96        | 1693.29    | 4.96        |

**Sup. Table 1. COVID-19 data [Cases per million (CpM) and deaths per million DpM] of European countries**

| Countries | Population | LTBI 2017 (%) |
|-----------|------------|---------------|
| Spain     | 46.47      | 7.06          |
| Iceland   | 34.15      | 7.07          |
| Finland   | 19.67      | 8.14          |
| Switzerland | 35.82   | 8.29          |
| Norway    | 147.38     | 8.84          |
| Belgium   | 34.40      | 9.84          |
| Denmark   | 116.30     | 10.03         |
| Portugal  | 7.65       | 10.33         |
| Czechia   | 10.92      | 11.41         |
| Estonia   | 30.50      | 12.88         |
| Turkey    | 3.85       | 13.62         |
| Slovakia  | 0.00       | 14.60         |
| Italy     | 0.819      | 16.46         |
| Hungary   | 0.846      | 18.90         |
| Lithuania | 1.081      | 20.88         |
| Bulgaria  | 0.071      | 23.06         |
| Ukraine   | 0.00       | 25.05         |

**Average (for countries <10 % LTBI)**

| Countries | Population | LTBI 2017 (%) |
|-----------|------------|---------------|
| UK        | 3.85       | 27.35         |
| Italy     | 1.081      | 33.08         |
| Hong Kong | 0.071      | 35.84         |
| Ukraine   | 0.00       | 37.14         |

1. Note: Countries name in Blue from ref 1.
2. CpM: Cases per million; DpM: Deaths per million; Mar: March; Apr: April; Jun: June; Jul: July; Aug: August 2020
3. LTBI estimates from ref 8. Global Burden of Disease Collaborative Network. Global Burden of Disease Study 2017 (GBD 2017) Results. Seattle, United States: Institute for Health Metrics and Evaluation (IHME), 2018. [Available from http://ghdx.healthdata.org/gbd-results-tool; search term combination "Prevalence - Latent Tuberculosis Infection - Sex: Both - Age: All Ages (Percent)"
4. StDev: Standard deviation

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**Average (for countries >10 % LTBI)**

| Countries | Population | LTBI 2017 (%) |
|-----------|------------|---------------|
| Spain     | 46.47      | 7.06          |
| Iceland   | 34.15      | 7.07          |
| Finland   | 19.67      | 8.14          |
| Switzerland | 35.82   | 8.29          |
| Norway    | 147.38     | 8.84          |
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| Countries | Population | LTBI 2017 (%) |
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| Hong Kong | 0.071      | 35.84         |
| Ukraine   | 0.00       | 37.14         |

**Notes:**
- LTBI: Latent Tuberculosis Infection
- Pop.: Population
- StDev: Standard deviation
Table 2. COVID-19 Cases and Deaths of erstwhile East and West Germany states (Ref.1)

### EAST GERMANY (Region)

| States/Dates | COVID-19 Cases per 100k population | COVID-19 Deaths per million population |
|--------------|-----------------------------------|----------------------------------------|
|              | 10-Apr 8-May 5-Jun 3-Jul 31-Jul | 10-Apr 8-May 5-Jun 3-Jul 31-Jul |
| Berlin: City State | 133.5 173.4 193.5 232.2 256.6 | 308.3 NA 44.42 54.5 58.32 60.23 60.77 |
| Brandenburg | 84.74 122.6 129.2 136.2 142 | 154.2 19.03 51.95 61.86 65.82 66.22 66.22 |
| Mecklenburg-Vorpommern [Mecklenburg-Western Pomerania] | 38.31 45.46 48.13 50 55.09 | 62.93 6.84 11.81 12.44 12.44 12.44 |
| Sachsen [Saxony] | 94.33 121.2 130.8 134.1 136.4 | 134.1 15.72 45.19 52.06 55.01 55.26 55.26 |
| Sachsen-Anhalt [Saxony-Anhalt] | 54.9 75.09 78.55 86.11 92.17 | 102.1 8.201 21.87 25.06 26.88 28.25 29.16 |
| Thüringen [Thuringia] | 73.55 122.3 145 153.5 158.6 | 169.7 11.25 52.5 79.22 84.84 85.31 85.31 |
| Average (without Berlin) | 65.27 91.01 100.62 105.93 110.57 120.48 | 10.50 32.84 42.20 44.79 45.32 45.54 |
| Average (with Berlin) | 79.89 110.01 120.86 132.02 140.14 157.41 | 12.21 37.96 47.52 50.55 51.29 51.53 |

### WEST GERMANY (Region)

| States/Dates | COVID-19 Cases per 100k population | COVID-19 Deaths per million population |
|--------------|-----------------------------------|----------------------------------------|
|              | 10-Apr 8-May 5-Jun 3-Jul 31-Jul | 10-Apr 8-May 5-Jun 3-Jul 31-Jul |
| Bremen: City State | 72.52 164.1 225.6 246.9 262.8 | 292 19.08 46.98 64.59 77.8 80.74 82.21 |
| Hamburg: City State | 218.2 269 275.7 281.8 294.5 | 340.6 28.69 108.8 137 140.7 140.7 141.3 |
| Baden-Württemberg | 235.7 301.5 315.5 323.2 337.6 | 378.5 55.31 136.5 160.2 165.7 166.4 |
| Bayern [Bavaria] | 264.7 340.3 361.5 371.7 389.7 | 437.2 54.93 161.1 189 197.9 199.3 199.8 |
| Hessen [Hesse] | 101.8 143.4 162.6 174.6 193.7 | 246.9 17.81 64.09 76.65 80.95 81.74 82.7 |
| Niedersachsen [Lower Saxony] | 102.8 136.7 156.7 171 182.1 | 209.8 21.39 61.67 75.56 79.19 80.56 81.44 |
| Nordrhein-Westfalen [North Rhine-Westphalia] | 150.1 195.8 215.7 245.8 275.7 | 327.8 27.97 77.84 90.1 93.94 95.34 96.56 |
| Rheinland-Pfalz [Rhineland-Palatinate] | 121.1 154.3 165.6 173 184.9 | 219.7 14.66 46.9 56.18 57.4 58.14 58.38 |
| Saarland | 209.2 263.4 272.9 277.6 285.7 | 309.7 NA 141.9 168.2 176.3 176.3 |
| Schleswig-Holstein | 76.76 101 107 110 119.9 | 139.6 NA 42.01 50.28 52.35 53.03 53.72 |
| Average (without Bremen & Hamburg) | 157.77 204.55 219.69 230.86 246.16 | 283.65 32.01 91.50 108.27 112.97 113.76 114.41 |
| Average (with Bremen & Hamburg) | 155.29 206.95 225.88 237.56 252.66 | 290.18 29.98 88.78 106.78 112.22 113.16 113.88 |

Reference 1. https://www.citypopulation.de/en/germany/covid/ [Accessed on 10 October 2020].
Table 3. i-CFR rates in East and West Germany region/states

| EAST GERMANY (Region)                  | i-CFR Rates at Indicated Dates | 8-May | 5-Jun | 3-Jul | 31-Jul | 28-Aug |
|--------------------------------------|---------------------------------|-------|-------|-------|--------|--------|
| Berlin: City State                   |                                 | 2.562 | 2.817 | 2.512 | 2.347  | 1.971  |
| Brandenburg                          |                                 | 4.237 | 4.788 | 4.833 | 4.663  | 4.294  |
| Mecklenburg-Vorpommern [Mecklenburg-Western Pomerania] |                     | 2.598 | 2.585 | 2.488 | 2.258  | 1.977  |
| Sachsen [Saxony]                     |                                 | 3.729 | 3.980 | 4.102 | 4.051  | 3.754  |
| Sachsen-Anhalt [Saxony-Anhalt]       |                                 | 2.913 | 3.190 | 3.122 | 3.065  | 2.856  |
| Thüringen [Thuringia]                |                                 | 4.293 | 5.463 | 5.527 | 5.379  | 5.027  |
| Average (w/o Berlin)                 |                                 | 3.609 | 4.194 | 4.229 | 4.098  | 3.780  |
| Average (with Berlin)                |                                 | 3.450 | 3.932 | 3.829 | 3.659  | 3.274  |
| WEST GERMANY                          |                                 |       |       |       |        |        |
| Bremen: City State                   |                                 | 2.863 | 2.863 | 3.151 | 3.072  | 2.815  |
| Hamburg: City State                  |                                 | 4.045 | 4.969 | 4.993 | 4.778  | 4.149  |
| Baden-Württemberg                    |                                 | 4.527 | 5.078 | 5.127 | 4.908  | 4.396  |
| Bayern [Bavaria]                     |                                 | 4.734 | 5.228 | 5.324 | 5.114  | 4.570  |
| Hessen [Hesse]                       |                                 | 4.469 | 4.714 | 4.636 | 4.220  | 3.350  |
| Niedersachsen [Lower Saxony]         |                                 | 4.511 | 4.822 | 4.631 | 4.424  | 3.882  |
| Nordrhein-Westfalen [North Rhine-Westphalia] |                     | 3.975 | 4.177 | 3.822 | 3.458  | 2.946  |
| Rheinland-Pfalz [Rhineland-Palatinate] |                                 | 3.040 | 3.393 | 3.318 | 3.144  | 2.657  |
| Saarland                             |                                 | 5.387 | 6.163 | 6.351 | 6.171  | 5.693  |
| Schleswig-Holstein                   |                                 | 4.159 | 4.699 | 4.759 | 4.423  | 3.848  |
| Average (w/o Bremen & Hamburg)       |                                 | 4.473 | 4.928 | 4.893 | 4.621  | 4.034  |
| Average (w/ Bremen & Hamburg)        |                                 | 4.290 | 4.727 | 4.724 | 4.479  | 3.924  |

Note: East Germany states (est. 22.5% LTBI of vulnerable population (Ref. 2)) consistently reported lower i-CFR as compared to West Germany States (est. 9.2% LTBI of vulnerable population). E. Germany with higher trained immunity correlate consistently had 20-30% lower CFR as compared to W. Germany states. The inclusion of Berlin in East Germany region, and that of Hamburg and Bremen in West Germany region decreased the closing trend of the i-CFR with passage of time (Compare covariation of Red and Green trend lines with Orange and Light green in Fig. 2C) that could be reflective of more LTBI positives in Berlin as compared to Hamburg and Bremen.

Reference 2. Singh S, Maurya RP, Singh RK. ‘Trained immunity’ from *Mycobacterium* spp. exposure or BCG vaccination and COVID-19 outcomes. medRxiv. DOI:10.1101/2020.07.11.20151308.