SARS-CoV-2 lineage B.1.1.7. Int J Legal Med. 2021;135:2347–9. PubMed https://link.springer.com/article/10.1007/s00414-021-02691-z
9. Walsh KA, Jordan K, Clyne B, Rohde D, Drummond L, Byrne P, et al. SARS-CoV-2 detection, viral load and infectivity over the course of an infection. J Infect. 2020;81:357–71. https://doi.org/10.1016/j.jinf.2020.06.067
10. Mohandas S, Yadav PD, Sapkal G, Shete AM, Deshpande G, Nyayanit DA, et al. Pathogenicity of SARS-CoV-2 Omicron (R346K) variant in Syrian hamsters and its cross-neutralization with different variants of concern. EBioMedicine. 2022;79:103997. https://doi.org/10.1016/j.ebiom.2022.103997

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Possible Mpox Protection from Smallpox Vaccine–Generated Antibodies among Older Adults

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DOI: https://doi.org/10.3201/eid2903.221231

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Smallpox vaccination may confer cross-protection to mpox. We evaluated vaccinia virus antibodies in 162 persons ≥50 years of age in Spain; 68.5% had detectable antibodies. Highest coverage (78%) was among persons 71–80 years of age. Low antibody levels in 31.5% of this population indicates that addressing their vaccination should be a priority.

As the 2022 mpox outbreak spread worldwide, protection against smallpox has become a focus of interest because smallpox vaccination might provide some protection against monkeypox virus (*1*). Massive vaccination with live vaccinia virus vaccines was conducted in most countries before smallpox was eradicated in 1980 (*2*), meaning a substantial proportion of persons ≥50 years of age as of 2022 might be protected against both diseases. One suggested approach to mpox protection during the current outbreak has been to administer smallpox vaccine to close contacts of infected persons (*3*,*4*). However, before taking this approach if the outbreak spreads to additional persons, concerns need to be addressed about whether smallpox vaccination provides real cross-protection and, if so, whether protection has waned over time.

We conducted a serologic study among 162 persons ≥50 years of age in Spain who had probably received smallpox vaccination to determine the seroprevalence of vaccinia virus antibodies (VVAbs). We included 10 unvaccinated persons <40 years of age as controls, avoiding persons 40–49 years of age to eliminate possible interference in findings from persons of those ages possibly having been immunized against smallpox in the final years of vaccination. Our aim was to ascertain the presence of residual vaccinia virus immunity among adult/elderly persons. The study was approved by the ethics committee of the Eastern Health Area of Valladolid (cod: PI 22–2798) and research performed according to the Declaration of Helsinki. We obtained written informed consent from participants before sampling.

We used the Anti-Vaccinia virus IMV/Envelop protein/H3L/p35 IgG ELISA (Alpha Diagnostic International, https://www.4adi.com) to detect IgG against the vaccinia envelope protein H3L/p35, following manufacturer specifications (Appendix, https://wwwnc.cdc.gov/EID/article/29/3/22-1231-App1.pdf). VVAb levels were expressed in units per milliliter. We stratified results by age group: 50–60, 61–70, 71–80, and >80 years.

Seroprevalence differed by age group. We found no VVAbs among the control group. Seroprevalence increased with age, until it dropped dramatically

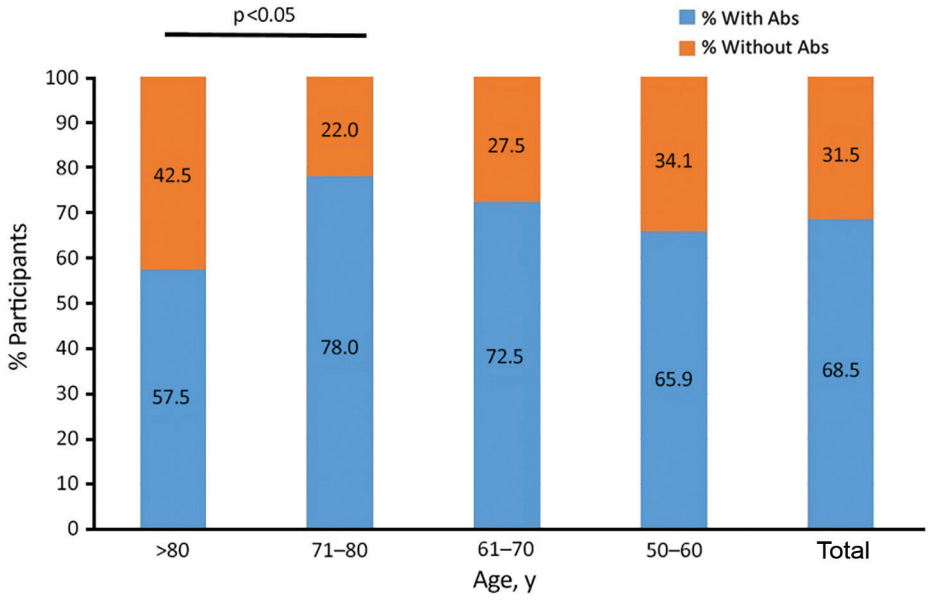

Figure. Seroprevalence of smallpox vaccine–generated antibodies among older adults, Spain. Detectable vaccinia virus antibody levels in the different age groups analyzed and for the total study population are given.

among participants >80 years of age (Figure). The 71–80 year age group exhibited the highest seroprevalence (78.0%), the >80 year group the lowest (57.5%) ($p < 0.05$ by χ^2 test). We found no significant differences in median VVAbs levels between the other age groups.

The relevance of these findings is that, 42 years after the end of routine smallpox vaccination in Spain, 68.5% of persons ≥50 years of age that we tested had detectable antibodies to vaccinia. That the highest seroprevalence was among participants in the 71–80-year age group and gradually decreased among younger age groups is probably explained by declines in smallpox vaccination coverage in Europe over time, rather than by decreased immune response.

Although guidelines for recommended smallpox vaccination did not change during 1937–1980 in most countries in Europe, vaccination coverage in Spain and other countries declined continuously as disease eradication progressed (*5*,*6*). For example, a 2019 article reported that smallpox vaccination coverage in Guinea-Bissau fell dramatically during the 1970s, from 75% to 10%–25% (*7*). Another study, conducted in Denmark, reported that vaccination coverage dropped from 95% in 1965 to 5%–20% among persons born during the 1970s (*8*). In Spain, >6 million smallpox vaccinations were administered in 1961 but only 725,371 in 1970 and 105,573 in 1979 (*5*,*6*). Furthermore, endemic cases in high-income countries declined greatly during the 1950s (*9*). Taken together, those data illustrate that smallpox vaccination coverage steadily declined in most high-income Western countries as smallpox was increasingly confined to low-income countries (*7*). Although an imported outbreak in Yugoslavia in 1972 caused 175 cases and 35 deaths, the last non-imported case in Europe was declared in 1953 (*10*); after that date, persons became less likely to receive smallpox vaccination.

The main limitation of our study is that we did not know the vaccination status of participants and thus could not determine whether lack of VVAbs was because of absence of vaccination or waning of antibodies. In addition, VVAbs levels might not correlate with immune protection against other orthopox viruses. The low number of participants might have affected statistical differences in results between groups. Finally, the absence of conserved cells precluded analysis of cellular immunity.

Our findings suggest that a substantial percentage (31.5%) of persons in Spain born before 1972, especially persons born during the years when routine smallpox vaccination use waned, have either not been vaccinated against smallpox or have lost the VVAbs induced by the vaccine. Assuming 85% maximum cross-protection against monkeypox virus conferred by smallpox vaccination (*1*) and 68.5% of the population ≥50 years of age having detectable VVAbs, we estimated that only 58.2% of persons in those age groups would be protected. Through September 2022, a total of 813 (12.4%) mpox cases in Spain had been reported in persons ≥50 years of age (*11*). Limited vaccine coverage might be one cause of these cases, so vaccination against mpox or with new smallpox vaccines should be a priority in this population.

I.S.M., R.O.L., J.C.S., and J.M.E. designed the study; J.S.M., L.S.D.P., S.R.R., and M.D.D.G. performed the experiments; I.S.M., L.S.D.P., C.H.G., and V.F.E. analyzed the data; I.S.M., L.S.D.P., R.O.L., J.C.S., and J.M.E. wrote the manuscript; R.O.L., J.C.S., and J.M.E. revised the manuscript; all authors edited and revised the final version of the manuscript.

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References

1. Centers for Disease Control and Prevention. Interim clinical considerations for use of JYNNEOS and ACAM2000 vaccines during the 2022 U.S. mpox outbreak [cited 2022 Jun 21]. https://www.cdc.gov/poxvirus/monkeypox/clinicians/smallpox-vaccine.html
2. Centers for Disease Control and Prevention. Smallpox [cited 2022 Jul 20]. https://www.cdc.gov/smallpox/index.html
3. World Health Organization. Multi-country monkeypox outbreak in non-endemic countries. 2022 [cited 2022 Jul 20]. https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON385
4. European Centre for Disease Prevention and Control. Monkeypox multi-country outbreak—first update [cited 2022 Jul 20]. https://www.ecdc.europa.eu/sites/default/files/documents/Monkeypox-multi-country-outbreak-first-update-8-July-FINAL3.pdf
5. Carlos III Health Institute. Analysis of healthcare in Spain throughout the 20th century [in Spanish] [cited 2022 Jul 26]. http://gesdoc.isciii.es/gesdoccontroller?action=download&id=19/10/2012-3c0cfd4ca3#page=228
6. Comité Asesor de Vacunas de la Asociación Española de Pediatría. Vaccination against smallpox in Spain in the years prior to its eradication [in Spanish] [cited 2022 Jul 26]. https://vacunasaep.org/profesionales/noticias/la-vacunacion-contra-la-viruela-en-espana-en-los-anos-anteriores-su-erradicacion
7. Rieckmann A, Villumsen M, Hønge BL, Sørup S, Rodrigues A, da Silva ZJ, et al. Phase-out of smallpox vaccination and the female/male HIV-1 prevalence ratio: an ecological study from Guinea-Bissau. BMJ Open. 2019; 9:e031415. https://doi.org/10.1136/bmjopen-2019-031415
8. Sørup S, Villumsen M, Ravn H, Benn CS, Sørensen TIA, Aaby P, et al. Smallpox vaccination and all-cause infectious disease hospitalization: a Danish register-based cohort study. Int J Epidemiol. 2011;40:955–63. https://doi.org/10.1093/ije/dyr063
9. Belongia EA, Naleway AL. Smallpox vaccine: the good, the bad, and the ugly. Clin Med Res. 2003;1:87–92. https://doi.org/10.3121/cmr.1.2.87
10. Centers for Disease Control and Prevention. History of smallpox [cited 2022 Jul 26]. https://www.cdc.gov/smallpox/history/history.html
11. Ministerio de Sanidad. Carlos III Health Institute. Epidemiological situation of monkeypox cases in Spain [in Spanish] [cited 2022 Oct 31]. https://www.isciii.es/QueHacemos/Servicios/VigilanciaSaludPublicaRENAVE/EnfermedadesTransmisibles/Documents/archivos%20A-Z/MPOX/SITUACION%20EPIDEMIOLOGICA%20DE%20LOS%20CASOS%20DE%20VIRUELA%20DEL%20MONO-30082022.pdf

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SARS-CoV-2 Infection in a Hippopotamus, Hanoi, Vietnam

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DOI: https://doi.org/10.3201/eid2903.220915

While investigating the death of a hippopotamus at a zoo in Hanoi, Vietnam, we isolated SARS-CoV-2 and sequenced the RNA-dependent RNA polymerase gene from different organs. Phylogenetic analysis showed that the SARS-CoV-2 strain was closely related to 3 human SARS-CoV-2 strains in Vietnam.

On December 4, 2021, a 20-year-old female hippopotamus (*Hippopotamus amphibius*) at a zoo in Hanoi, Vietnam, was treated for lethargy, depression, and reduced appetite. Veterinary staff initiated antimicrobial drug treatment on the basis of the clinical signs.

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Article DOI: https://doi.org/10.3201/eid2903.221231

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Appendix

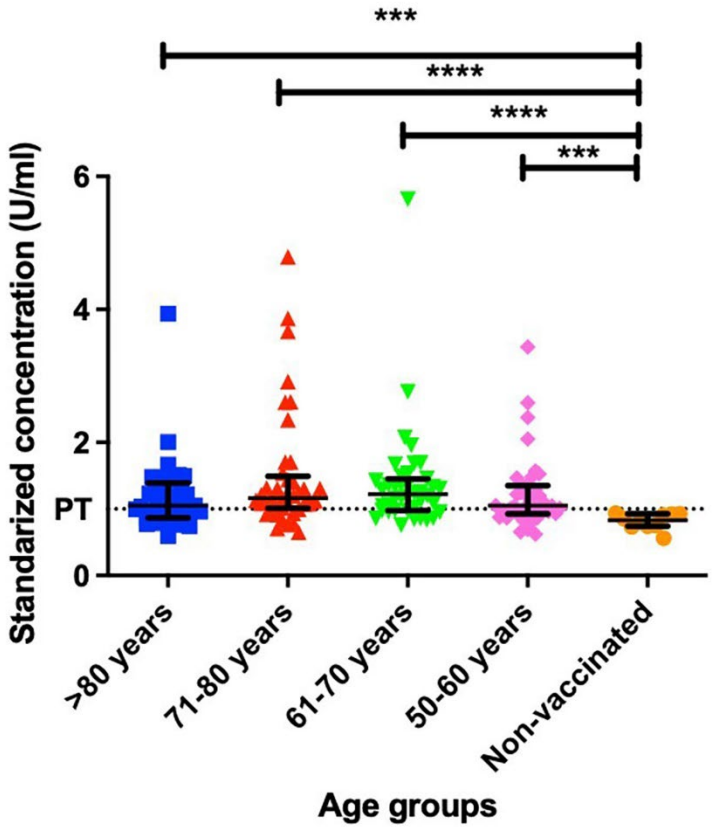
ELISA Method Used for Detecting and Quantifying Antibodies against Vaccinia Virus

A detection analysis for specific antibodies against Vaccinia poxvirus was performed to detect and quantify the antibodies against the Vaccinia virus present in the serum samples persons included in the study. To perform this analysis, the Human Anti-Vaccinia Virus (VACV) IMV/Envelope Protein/H3L/p35 IgG ELISA Kit reagents (Alpha Diagnostic International, https://www.4adi.com) were used, which enable the detection and quantification of IgG antibodies against the vaccine envelope protein H3L/p35 of the intracellular mature virion (IMV), which is the most abundant infectious protein of Orthopoxviruses. Additionally, to the serum samples included in the study, a positive and negative control (supplied with the reagents) and four calibrators (H3L positives at different concentrations) were included in duplicate to evaluate the level of vaccinia virus antibodies (VVAb) present in the positive samples.

Briefly, this method is an ELISA where the antibodies present in the serum bind to the H3L/p35 protein that coats the wells. After an initial dilution of the serum following the manufacturer's specifications (1:50), then were incubated for 60 minutes at room temperature and subsequently four washes performed. A second incubation was performed in which the secondary Anti-Human IgG HRP antibody was added and incubated at room temperature for 30 minutes. Then, the TMB chromogenic substrate (tetramethylbenzidine) was added and incubated for 15 minutes in the dark. Following this, the stop solution was added to wells and read on a plate reader at 450nm wavelength.

Reading the results was following the manufacturer´s protocol. Briefly, a standard curve was made using the duplicate values of the calibrators (1 units/mL [U/mL], 2.5 U/mL, 5 U/mL,

and 10 U/mL), expressing the antibody levels of each serum in U/mL. According to manufacturer's instructions, the value 1 U/mL was the positive threshold. Thus, those antibody values above 1 U/mL were considered positive and below as negative. The nonvaccinated controls were used to validate the established 1 U/mL cutoff value of the technique.



Appendix Figure. Seroprevalence of smallpox vaccine–generated antibodies among older adults, Spain. Median (interquartile range) of the standardized VVAb levels (U/mL) in each age group and in persons <40 years of age (controls) are shown. Blue boxes, >80 years of age; red upward triangles, 71–80 years of age; green downward triangles, 61–70 years of age; purple diamonds, 50–60 years of age; orange dots, nonvaccinated <40 years of age. Abs, antibodies; PT, positive threshold. *p<0.05; ***p<0.001; ****p<0.0001.