Supplementary Appendix

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This appendix has been provided by the authors to give readers additional information about the work.

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1.Supplementary Discussion

In this study, prior vaccination with a first (Dryvax®) or second (ACAM2000®) generation smallpox vaccine was effective in preventing mpox among military personnel and Veterans with an estimated vaccine effectiveness (VE) of 74% and 66% respectively. This is the first and largest real-world study estimating the effectiveness of older generation smallpox vaccines against mpox, including individuals with a more remote vaccination history.

Surveillance data from a historical outbreak in the Democratic Republic of Congo estimated a protective effect (85% risk reduction) of prior smallpox vaccination with Dryvax® against mpox, among household contacts of individuals with the disease(1, 2). This report was limited by a small sample size and likely does not reflect the level of protection conferred by updated smallpox vaccines. In contrast, our study evaluated the effectiveness of smallpox vaccination received between 2002-2017, which is within 20 years of the current outbreak. Comparatively, our effectiveness estimates are lower, suggesting differences in the level of protection conferred likely exist, based upon the vaccine formulation used or time since vaccination. Furthermore, the role of mucosal transmission in the current mpox outbreak may differentially modulate the effectiveness of vaccines administered by subcutaneous and intradermal routes. Understanding how novel modes of transmission impact the efficacy of available vaccines requires investigation.

The odds of testing positive for mpox decreased with increasing age in our cohort, independent of documented smallpox vaccination. This mirrors patterns observed with other sexually transmissible infections such as gonorrhea, chlamydia, and syphilis and likely reflects changes in sexual behavior patterns that may occur with older age(3).

It is important to highlight that protection against mpox conferred by previous smallpox vaccination is not absolute, and vaccines need to be combined with other infection prevention strategies. While vaccine-induced immune responses following Dryvax® or ACAM2000® vaccination may provide durable protection against mpox, a subset of individuals, yet to be defined, may benefit from subsequent booster doses to optimize this protection. Current CDC guidance recommends a booster for individuals with continued exposure to *Orthopoxviruses* two-years after a primary series of JYNNEOS® vaccine and three-years after the single dose of

ACAM2000®(4). The need for booster doses of vaccine and the timing of these for mpox remains to be determined.

In our cohort, HIV diagnosis and Male sex were associated with an increased likelihood for acquiring mpox. In the US 98% of mpox cases are in men who have sex with men, and 38% have concomitant infection with HIV(5). Black and Hispanic ethnicities were associated with increased odds for mpox. Similar observations have been made in other studies, highlighting emerging disparities in the burden of mpox among people with HIV and individuals from racial and ethnic minority groups in the US. These emerging risk associations should be considered in prioritizing access to prevention tools against mpox for military personnel.

Limitations - The retrospective test-negative, non-randomized design increases the potential for selection bias i.e., individuals who sought care and testing may have different characteristics from those who did not seek care for their symptoms. This could potentially affect the accuracy of VE estimates based only on individuals who were tested for orthopoxvirus. Individuals diagnosed with HIV prior to separation from the military may have been less likely to be vaccinated in the military because of their restriction from deployment and for safety reasons. This introduces a potential indication bias and may over-estimate VE especially if this group would have had a lower VE if vaccinated. However, the vast majority of Veterans were diagnosed with HIV after separation, which would not have affected the decision to vaccinate. Our cohort included Veterans and current military personnel who have access to healthcare through the VA and the DoD. As such we are cautious in applying our findings to a general population with different risk characteristics, healthcare care seeking behavior and health care access profiles. Finally, residual confounding factors such a history of pediatric smallpox vaccination in a small proportion of older study participants, for which we have not fully accounted, could potentially affect the accuracy of the VE estimates.

Conclusions

The re-emergence of mpox poses a threat to populations everywhere. In combination with other public health interventions, smallpox vaccines can reduce the spread of mpox virus. Evaluating

the effectiveness of smallpox vaccines against mpox is essential, while also ensuring that all who can potentially benefit from them have equitable access globally.

2. Study Plan and Design - Effectiveness of ACAM2000® and DRYVAX® for Preventing Monkeypox among the VA and DoD population

a) Rationale

Over 40,000 cases of Monkeypox have across the United States in recent months. Two vaccines have been used for the prevention of monkeypox, ACAM2000 (2nd generation smallpox vaccine) and JYNNEOS (3rd generation smallpox vaccine). The most frequently cited data regarding the effectiveness of smallpox vaccines against monkeypox is from a retrospective analysis published in 1988 which examined whether smallpox vaccination with Dryvax (1st generation smallpox vaccine) could also prevent mpox and reported an effectiveness of 85% against monkeypox among 338 patients in the Democratic Republic of Congo. However, there are limited data currently available on the clinical efficacy or effectiveness of older generation vaccines DRYVAX and ACAM2000 in the current outbreak. Additionally, it is unclear how much the effectiveness of vaccination wanes over time or whether boosting previously vaccinated individuals is beneficial.

b) Hypothesis – We hypothesized that prior vaccination with a smallpox vaccine is protective against mpox disease.

c) Study population

The VA provides care to nearly 9 million Veterans at 171 medical centers and 1112 outpatient clinics across the US and has been instrumental in providing information regarding real-word vaccine effectiveness during the COVID-19 pandemic. In 1972, routine smallpox vaccination was discontinued after the disease was eradicated in the United States. In 2021, nearly 73% of Veterans were at least 50 years old and eligible to have been vaccinated in childhood. More than 2.6 million service members received the ACAM2000 smallpox vaccine between 2002 and 2017 as a pre-deployment requirement or for occupational reasons. Most of these individuals have since become Veterans. About 60% of all Veterans have used a Veterans Health Administration (VHA) health care center, representing a substantial population of individuals who may have

received the ACAM2000 vaccine in this millennium. Since the mpox outbreak began in the U.S., individuals with perceived risk of infection have obtained either the ACAM2000 or JYNNEOS vaccine through local departments of health. Using a combined cohort of active military service men within the DoD and VHA provides an opportunity to estimate the effectiveness of prior smallpox vaccination against mpox in ongoing outbreaks of the disease.

d) Study design and statistical analysis plan

We conducted a retrospective observational test-negative case-control evaluation of vaccine effectiveness by comparing the odds of antecedent vaccination in cases (individuals with PCR-test confirmed mpox) vs. control (test-negative individuals without mpox). Cases patients were selected based on positive results for non-variola Orthopoxvirus generic real-time PCR testing. All study participants were drawn from individuals with 'Medically attended' symptoms) of mpox defined as persons who sought medical attention for or were identified by a clinician as having clinical syndromes concerning for mpox and underwent orthopoxvirus testing as part of a diagnostic evaluation within the DoD or the VHA healthcare systems.

ACAM2000 and Dryvax administration was captured using VA and DoD immunization records. Everyone included in the study population was vaccinated by the military and in conjunction with military service. The individuals included in the VA population enrolled in the VHA post active duty and had their follow-up here . We have included this in the description of the study population. We excluded individuals with remote smallpox vaccination (e.g., pediatric age) which we could not verify using DoD or VHA health records.
Other covariates included age, race/ethnicity, comorbidities, time since vaccination, HIV diagnosis and CD4 recovery, and plasma HIV RNA viral load suppression. We conducted a retrospective analysis comparing the rate of vaccination among test positive individuals and test negative controls in the combined VHA/DoD cohort and estimated rates of monkeypox by vaccine type received for the following sub-categories

a. in VA/DoD population overall and in each sub-population separately
b. among monkeypox cases overall
c. among mpox negative controls overall

d. among people with HIV overall

e. monkeypox cases with HIV

f. monkeypox negative controls with HIV

We determined the odds of vaccination among cases compared with controls and calculated vaccine effectiveness estimates (1− OR of mpox in vaccinated/OR of mpox in unvaccinated x 100%) adjusting for covariates including age, race/ethnicity, and HIV status.

3. Supplementary Tables

Supplementary Table S1: Demographic and clinical characteristics of individuals tested for Non-variola *Orthopoxvirus* (NVO) across DoD and VHA (July 1 – October 31, 2022)

		Total		Negative for NVO		Positive NVO	
		N	%	N	%	N	%
Population		1,007	100%	709	70%	298	30%
Age	Median	43		49		37	
Age							
	Age 18-34	308	31%	182	26%	126	42%
	Age 35-44	216	21%	136	19%	80	27%
	Age 45-64	339	34%	252	36%	87	29%
	Age 65+	144	14%	s	s	s	s
Sex							
	Female	77	8%	s	s	s	s
	Male	930	92%	635	90%	295	99%
Race							
	Black	410	41%	268	38%	142	48%
	Hispanic	123	12%	82	12%	41	14%
	White	376	37%	283	40%	93	31%
	Other or unknown	98	10%	76	11%	22	7%
Data source							
	DoD data	209	21%	102	14%	107	36%
	VHA data	798	79%	607	86%	191	64%
Group							
	DoD (service member)	198	20%	97	14%	101	34%
	DoD (Veteran)	11	1%	s	s	s	s
	VHA (Veteran)	798	79%	607	86%	191	64%
Vaccinated							
	No	799	79%	540	76%	259	87%
	Yes	208	21%	169	24%	39	13%
Vaccine type							
	None	799	79%	540	76%	259	87%

	ACAM2000	125	60%	101	60%	24	62%
	Dryvax	83	40%	68	40%	15	38%
Number of ACAM shots							
	0	882	88%	608	86%	274	92%
	1	117	12%	96	14%	21	7%
	2	s	s	s	s	s	s
	3+	-	0%	-	0%	-	0%
Number of Dryvax shots							
	0	915	91%	633	89%	282	95%
	1	75	7%	64	9%	11	4%
	2	11	1%	s	s	s	s
	3+	s	s	s	s	s	s
Both vaccine types (People with at least one ACAM and at least one Dryvax)		s	s	s	s	s	s
Number of shots							
	0	799	79%	540	76%	259	87%
	1	178	18%	148	21%	30	10%
	2+	30	3%	s	s	s	s
Years since vaccination							
	None	799	79%	540	76%	259	87%
	0 to 4	18	9%	s	s	s	s
	5 to 10	37	18%	s	s	s	s
	11 to 15	92	44%	74	44%	18	46%
	16+	61	29%	s	s	s	s
Diagnosed with HIV							
	No	712	71%	545	77%	167	56%
	Yes	295	29%	164	23%	131	44%
Incident hospitalization							
	Missing	209	21%	102	14%	107	36%
	No	780	77%	603	85%	177	59%
	Yes	18	2%	s	s	s	s

S = suppressed (– we followed the Medicare rules for data suppression for VHA and DoD datasets)

Supplementary Table S2: Vaccination status of study population by vaccine type, number of doses and years since vaccination -DoD and VHA (July 1 – October 31, 2022)

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		TOTAL		DoD		VHA	
		N	%	N	%	N	%
Population		1,007	100.0%	209	20.8%	798	79.2%
Vaccinated							
	No	799	79.3%	170	81.3%	629	78.8%
	Yes	208	20.7%	39	18.7%	169	21.2%
Vaccine type							
	None	799	79.3%	170	81.3%	629	78.8%
	ACAM2000	125	60.1%	30	76.9%	95	56.2%
	Dryvax	83	39.9%	s	s	s	s
Number of ACAM2000 doses							
	0	882	87.6%	179	85.6%	703	88.1%
	1	117	11.6%	30	14.4%	87	10.9%
	2	s	s	s	s	s	s
	3 or more	-	0.0%	-	0.0%	-	0.0%
Number of Dryvax doses							
	0	915	90.9%	198	94.7%	717	89.8%
	1	75	7.4%	10	4.8%	65	8.1%
	2	11	1.1%	s	s	s	s
	3 or more	s	s	s	s	s	s
Number of overall vaccine doses regardless of sub-type							
	0	799	79.3%	170	81.3%	629	78.8%
	1	178	17.7%	37	17.7%	141	17.7%
	2 or more	30	3.0%	s	s	s	s
Years since vaccination							
	(none)	799	79.3%	170	81.3%	629	78.8%
	0 to 4	18	8.7%	s	s	s	s
	5 to 10	37	17.8%	12	30.8%	25	14.8%

	11 to 15	92	44.2%	13	33.3%	79	46.7%
	16+	61	29.3%	s	s	s	s

S = suppressed (– we followed the Medicare rules for data suppression for VHA and DoD datasets)

Supplementary Table S3: Regression models and outputs

Supplementary Table S3 – Fit of regression models

Model	Subpopulation	Criterion	Intercept Only	Intercept and Covariates	Included in model
1	**Hospitalized - tested positive (VHA data only)**	AIC	102.12	107.09	Vaccinated, age, race
		SC	105.37	123.35	
		-2 Log L	100.12	97.09	
2	**Full population**	AIC	1225.25	1211.43	Vaccinated
		SC	1230.17	1221.26	Vaccinated
		-2 Log L	1223.25	1207.43	Vaccinated
3	**Female**	AIC	27.35	28.02	Vaccinated
		SC	29.70	32.71	Vaccinated
		-2 Log L	25.35	24.02	Vaccinated
4	**Male**	AIC	1164.02	1150.16	Vaccinated
		SC	1168.86	1159.83	Vaccinated
		-2 Log L	1162.02	1146.16	Vaccinated
5	**Race - Black**	AIC	531.02	528.09	Vaccinated
		SC	535.04	536.13	Vaccinated
		-2 Log L	529.02	524.09	Vaccinated
6	**Race - Hispanic**	AIC	158.58	156.95	Vaccinated
		SC	161.39	162.58	Vaccinated
		-2 Log L	156.58	152.95	Vaccinated
7	**Race - White**	AIC	422.66	421.64	Vaccinated
		SC	426.59	429.50	Vaccinated

		-2 Log L	420.66	417.64	Vaccinated
8	**Race - Other/Unknown**	AIC	106.38	101.73	Vaccinated
		SC	108.96	106.90	Vaccinated
		-2 Log L	104.38	97.73	Vaccinated
9	**Not diagnosed with HIV**	AIC	777.68	766.78	Vaccinated
		SC	782.24	775.91	Vaccinated
		-2 Log L	775.68	762.78	Vaccinated
10	**HIV positive**	AIC	407.26	406.55	Vaccinated
		SC	410.94	413.92	Vaccinated
		-2 Log L	405.26	402.55	Vaccinated
11	**Full population**	AIC	1225.25	1012.68	Vaccination type, age, race, diagnosed with HIV, male, DoD data
		SC	1230.17	1071.66	
		-2 Log L	1223.25	988.68	
12	**Vaccination type**	AIC	1225.25	1213.39	Vaccination type
		SC	1230.17	1228.13	
		-2 Log L	1223.25	1207.39	
13	**Age**	AIC	1225.25	1139.05	Age
		SC	1230.17	1158.71	
		-2 Log L	1223.25	1131.05	
14	**Race**	AIC	1225.25	1218.65	Race
		SC	1230.17	1238.31	
		-2 Log L	1223.25	1210.65	
15	**Diagnosed with HIV (yes/no)**	AIC	1225.25	1184.93	Diagnosed with HIV
		SC	1230.17	1194.76	
		-2 Log L	1223.25	1180.93	
16	**Male**	AIC	1225.25	1191.38	Male
		SC	1230.17	1201.21	
		-2 Log L	1223.25	1187.38	
17	**Under 65**	AIC	1107.87	966.94	Vaccination type, age, race, diagnosed with HIV, male, DoD data

		SC	1112.63	1019.30	
		-2 Log L	1105.87	944.94	
18	**HIV positive**	AIC	407.26	380.12	Vaccinated, age, race, DoD data
		SC	410.94	409.62	
		-2 Log L	405.26	364.12	
19	**Not diagnosed with HIV**	AIC	777.68	640.44	Vaccinated, age, race, DoD data
		SC	782.24	681.55	
		-2 Log L	775.68	622.44	
20	**Vaccinees**	AIC	202.75	192.45	Diagnosed with HIV, age, race, DoD data
		SC	206.09	219.15	
		-2 Log L	200.75	176.45	
21	**0-5 years since vaccination**	AIC	1030.19	844.44	Vaccinated, diagnosed with HIV, age, race, male, is DoD
		SC	1034.90	891.53	
		-2 Log L	1028.19	824.44	
22	**0-5 years since vaccination**	AIC	1030.19	1030.70	Vaccinated
		SC	1034.90	1040.11	
		-2 Log L	1028.19	1026.70	
23	**6-10 years since vaccination**	AIC	1046.29	851.94	Vaccinated, diagnosed with HIV, age, race, male, is DoD
		SC	1051.01	899.20	
		-2 Log L	1044.29	831.94	
24	**6-10 years since vaccination**	AIC	1046.29	1045.71	Vaccinated
		SC	1051.01	1055.16	
		-2 Log L	1044.29	1041.71	
25	**11+ years since vaccination**	AIC	1082.14	887.32	Vaccinated, diagnosed with HIV, age, race, male, is DoD
		SC	1086.90	935.00	
		-2 Log L	1080.14	867.32	
26	**11+ years since vaccination**	AIC	1082.14	1077.87	Vaccinated
		SC	1086.90	1087.41	

		-2 Log L	1080.14	1073.87	

These contingency tables with estimated VE for each population (DoD and VA) are now included in the supplementary material.

	DoD		
	OPXV PCR		
Smallpox vaccine	Positive	Negative	Total
Yes			39
No			170
Total	107	102	209

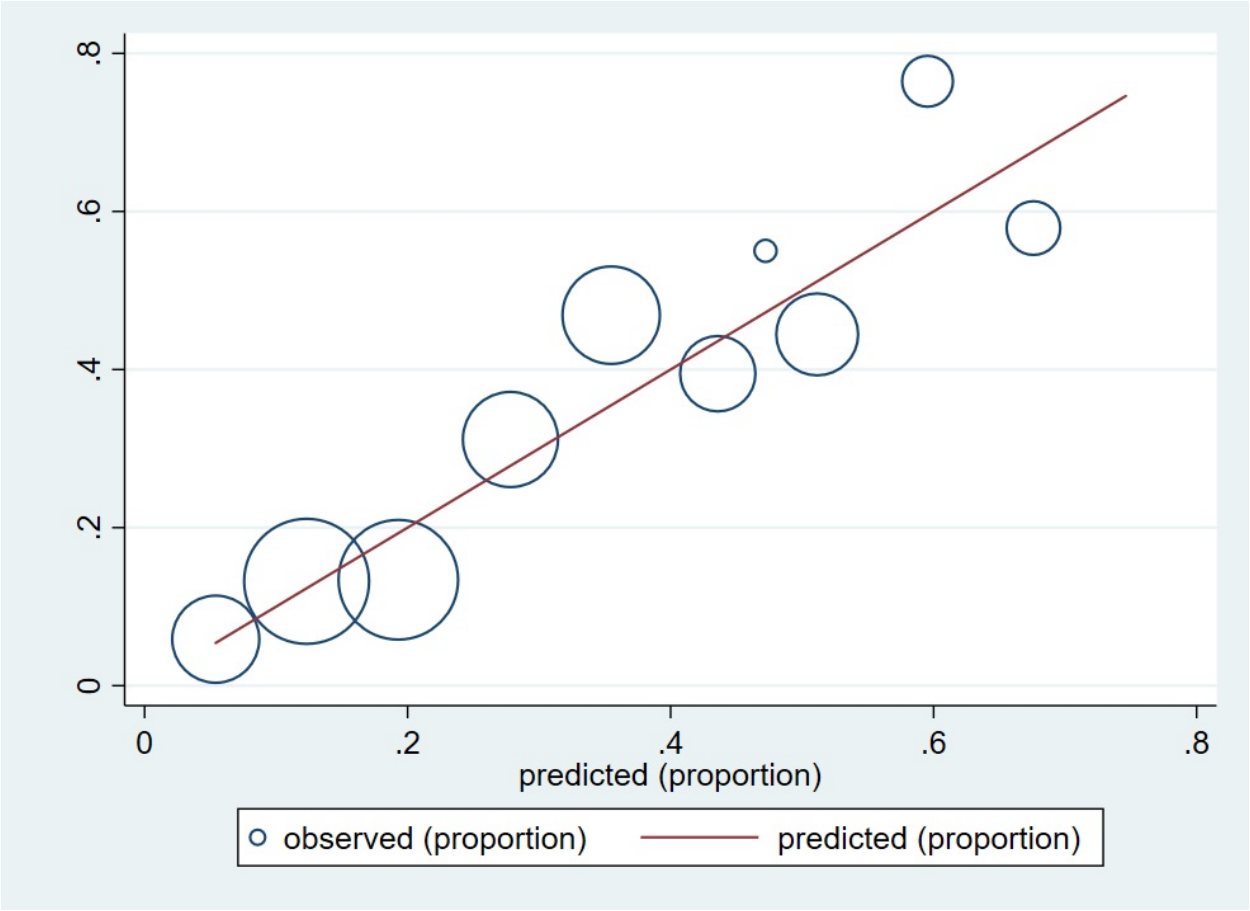
DoD - OR = xxx ; VE estimate = xxx

	VHA		
	OPXV PCR		
Smallpox vaccine	Positive	Negative	Total
Yes			169
No			629
Total	191	607	798

VHA – OR = xxx; VE estimate =

4. Supplementary Figures

Figure S1: Assessment of Model Fit using Hosmer-Lemeshow Method
Hosmer-Lemeshow chi2 (8) = 11.10
Prob > chi2 = 0.1961 (good fit)



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