

BMJ Open

BMJ Open is committed to open peer review. As part of this commitment we make the peer review history of every article we publish publicly available.

When an article is published we post the peer reviewers' comments and the authors' responses online. We also post the versions of the paper that were used during peer review. These are the versions that the peer review comments apply to.

The versions of the paper that follow are the versions that were submitted during the peer review process. They are not the versions of record or the final published versions. They should not be cited or distributed as the published version of this manuscript.

BMJ Open is an open access journal and the full, final, typeset and author-corrected version of record of the manuscript is available on our site with no access controls, subscription charges or pay-per-view fees (<http://bmjopen.bmj.com>).

If you have any questions on BMJ Open's open peer review process please email info.bmjopen@bmj.com

BMJ Open

Testing the hypothesis that the termination of smallpox vaccination may have increased the female/male HIV-1 prevalence ratio: an ecological study from Guinea-Bissau

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2019-031415
Article Type:	Research
Date Submitted by the Author:	07-May-2019
Complete List of Authors:	Rieckmann, Andreas; Bandim Health Project, Statens Serum Institut Villumsen, Marie Hønge, Bo; Aarhus University Hospital, Department of Infectious Diseases Sørup, Signe Rodrigues, Amabelia; Bandim Health Project, Bandim Health Project, da Silva, Zacarias Whittle, Hilton; London School of Hygiene and Tropical Medicine, Benn, Christine; Statens Serum Institut, Bandim Health Project; University of Southern Denmark, OPEN Aaby, Peter; Bandim Health Project,
Keywords:	Heterologous immunity, HIV-1, Non-specific effects of vaccines, Smallpox vaccination, Vaccinia

SCHOLARONE™
Manuscripts

Testing the hypothesis that the termination of smallpox vaccination may have increased the female/male HIV-1 prevalence ratio: an ecological study from Guinea-Bissau

Andreas Rieckmann^{1,2*}, Marie Villumsen³, Bo Langhoff Hønge^{4,5}, Signe Sørup^{1,6}, Amabélia Rodrigues⁵, Zacarias José da Silva⁷, Hilton Whittle⁸, Christine Stabell Benn^{1,9}, Peter Aaby⁵

¹ Research Center for Vitamins and Vaccines (CVIVA), Bandim Health Project, Statens Serum Institut, Artillerivej 5, DK-2300 Copenhagen S, Denmark (Andreas Rieckmann, PhD; Signe Sørup, PhD; Christine Stabell Benn, professor)

² Section of Epidemiology, Department of Public Health, University of Copenhagen, Copenhagen, Denmark (Andreas Rieckmann, PhD).

³ Center for Clinical Research and Prevention, Bispebjerg and Frederiksberg Hospital, Capital Region, Copenhagen, Denmark (Marie Villumsen, PhD)

⁴ Department of Clinical Immunology, Aarhus University Hospital, Denmark (Bo Langhoff Hønge, medical doctor)

⁵ Bandim Health Project, Indepth Network, Apartado 861, Bissau, Guinea-Bissau (Bo Langhoff Hønge, medical doctor; Amabélia Rodrigues, PhD; Peter Aaby, professor)

⁶ Department of Clinical Epidemiology, Aarhus University, Olof Palmes Allé 43-45, DK-8200 Aarhus N, Denmark (Signe Sørup, PhD).

⁷ National Institute of Public Health (INASA), CP 1013, Bissau, Guinea-Bissau (Zacarias J. da Silva, PhD, medical doctor)

⁸ London School of Hygiene and Tropical Medicine, Keppel Street, London, UK (Hilton Whittle, professor)

⁹ OPEN, Odense University Hospital/Institute of Clinical Research, University of Southern Denmark, Odense, Denmark (Christine Stabell Benn, professor)

1
2 * Corresponding author: Andreas Rieckmann, anri@ssi.dk
3

4 Word count: Abstract: 286, Manuscript 2756, References 22
5

6 Tables: 1, Figures: 2, Supplementary figures 2.
7

8
9 **Financial support:** The Danish National Research Foundation (DNRF) supports the Research
10 Center for Vitamins and Vaccines [DNRF108]. AR was supported by an unrestricted Faculty of
11 Health Sciences-scholarship from University of Southern Denmark. SS was supported by a grant
12 from the Danish Council for Independent Research [DFR – 4183-00316].
13
14
15
16

17
18 **Disclosures:** The authors have no conflicts of interest.
19

20
21 **Ethics statement:** Our study is based on published results from 3 original research papers. The
22 study by da Silva et al. was approved by the Guinea-Bissau Government Ethics Committee and the
23 Danish Central Scientific Ethics Committee. The study by van Tienen was approved by the Gambia
24 Government/MRC Laboratories Joint Ethics Committee and by the Ministry of Health of Guinea-
25 Bissau. The study by Olesen et al. was approved by the National Research Ethics Committee in
26 Guinea-Bissau and received consultative approval from the National Research Ethics Committee of
27 Denmark.
28
29
30
31
32
33
34
35

36
37 **Data access:** Information about HIV-1 was extracted from published results from original research
38 papers carried out in parallel both in Bissau (1987, 1996, 2006 [da Silva et al], 2016 [Olesen et al.])
39 and Caió (1990, 1997, 2007 [van Tienen et al.] in Guinea-Bissau. Information about smallpox
40 vaccination was based on data from a cohort of individuals, who had both participated in a smallpox
41 vaccination scar survey (2005-2007) and an HIV prevalence survey (2004-2006) conducted in
42 Bissau.
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Abstract

Objective: In Guinea-Bissau, West Africa, we observed that having a smallpox vaccination scar vs. not having it was associated with lower HIV-1 prevalence, more strongly for women. If this represents a causal effect, the female/male HIV-1 prevalence ratio would increase for birth cohorts no longer receiving smallpox vaccination due to the termination of this vaccine.

Design, Setting and participants: We compared aggregated data about smallpox vaccination coverage and results of sequential HIV surveys from two sites in Guinea-Bissau with long-term follow-up, between an age group with decreasing smallpox vaccination coverage (15-34 years) and an age group with steady smallpox vaccination coverage (≥ 35 years).

Results: At both study sites, the female/male HIV-1 prevalence ratio increased over time for the age group with decreasing smallpox vaccination coverage; the combined female/male HIV-1 prevalence ratio among 15-34-year-olds was 1.00 (95% confidence interval (CI) 0.17-5.99) in 1987-90, 1.16 (95% CI 0.69-1.93) in 1996-97, 2.32 (95% CI 1.51-3.56) in 2006-07 (p-value for no linear trend=0.04). There was no increase in the female-male HIV-1 prevalence ratio for the age group ≥ 35 years with steady smallpox vaccination coverage (1.93 (95% CI 0.40-9.25) in 1987-90, 1.32 (95% CI 0.83-2.10) in 1996-97, 0.81 (95% CI 0.56-1.16) in 2006-07 (p-value for no linear trend=0.07)).

Conclusion: Thus, data was compatible with the deduction that terminating smallpox vaccination may have increased the susceptibility to HIV-1 relatively more for women than for men. Hence, terminating smallpox vaccination may have contributed to the striking global increase in the female/male HIV-1 prevalence ratio among young individuals. Due to the potential fallacies of ecological studies, the results should be interpreted carefully and more research is needed to test this hypothesis. If the hypothesis is true, studies of smallpox vaccination could inform HIV-1 vaccine research.

1
2 Key words: Heterologous immunity; HIV-1; Non-specific effects of vaccines; Smallpox
3
4 vaccination; Vaccinia.
5
6
7
8
9

10 11 Article summary

12 13 **Strengths and limitations of this study**

- 14
15
16
17 • The ecological design allowed us to assess a deduction of the hypothesis: Smallpox vaccination has a protective
18 effect against HIV-1, which is stronger in women than men.
- 19
20
21 • To increase the robustness of the results, we utilized parallel data from both urban and rural Guinea-Bissau.
- 22
23 • Ecological studies should be interpreted carefully as spurious associations can arise, and thus triangulation with
24 existing studies are necessary.
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Introduction

Vaccination against smallpox infections was stopped globally in 1980 following the eradication of smallpox in 1977. It has been reported that smallpox vaccination reduced susceptibility to unrelated infectious diseases,(1) and in immunological *in vitro* studies, smallpox vaccination was associated with an up to 5-fold reduction in C-C chemokine receptor 5 (CCR5) tropic HIV-1 replication.(2) Based on vaccination scar readings in Guinea-Bissau and school health records in Denmark, we have shown that smallpox vaccination (and Bacille Calmette-Guérin vaccination [BCG]) was associated with a lower risk of HIV-1.(3) The adjusted odds ratio for HIV-1 infection was 0.52 (95% CI 0.32-0.84) for women and 0.77 (95% CI 0.48-1.24) for men. This association was stronger for women, who had received multiple smallpox vaccinations (odds ratio of 0.18 [95% CI, 0.05–0.64]).

We hypothesized that smallpox vaccination has a stronger protective effect against HIV-1 in women than men. If this is the case, the logical deduction is that the female/male HIV-1 prevalence ratio should increase for age groups with decreasing smallpox vaccination coverage while there would be no change in the female/male HIV-1 prevalence ratio for age groups with steady smallpox vaccination coverage. By using a female/male HIV-1 prevalence ratio, we could to some extent disregard time trends – such as the general spread of HIV-1 worldwide and the increased focus on prophylaxis and treatment – affecting both sexes and all age groups. We tested the hypothesis in two cohorts followed with sequential HIV surveys in Guinea-Bissau since the late 1980s.

Methods

This ecological study compared the changes in smallpox vaccination coverage with the change in female/male HIV-1 prevalence ratio for the age groups that were between 15-34 and ≥ 35 years over a 30-year period based on aggregated data from different sources. We used reported HIV-1 prevalence surveys in Bissau, the capital of Guinea-Bissau, and Caió, a rural district of Guinea-

1
2 Bissau.(4, 5) We used data from a smallpox vaccination scar survey in Bissau in 2005 to estimate
3
4 smallpox vaccination coverages at the time of the HIV-1 surveys.
5

6 **Patient and Public Involvement**

7
8 As this study was based on previously published data, patient and public were not involved in
9
10 conducting this research.
11
12

13 **Estimates of smallpox vaccination coverage**

14
15 Smallpox vaccination typically leaves a distinct vaccination scar. We used a cohort of individuals,
16
17 who had both participated in a smallpox vaccination scar survey (2005-2007) and an HIV
18
19 prevalence survey (2004-2006) conducted in Bissau (previously published (6)) to approximate the
20
21 historical changes in smallpox vaccination coverage. The smallpox vaccination scar prevalence is
22
23 comparable between urban and rural Guinea-Bissau.(7, 8) In the smallpox vaccination scar survey,
24
25 field workers examined vaccination scars and interviewed study participants. The field workers
26
27 examined upper arms for scars and registered all scars up to a maximum of five scars. Scars were
28
29 classified as BCG, smallpox vaccination, or “uncertain”, based on size, color, and general
30
31 appearance of the scar.
32
33
34
35
36
37
38

39 For each individual in the smallpox scar survey, we calculated the age the individual would have
40
41 had in the different HIV survey years. We approximated the age-standardized smallpox vaccination
42
43 coverage overall and by sex in the years 1987, 1996, 2005 and 2016 in each age group (15-34 and
44
45 ≥ 35 years) by dividing the number of individuals with a smallpox vaccination scar by the total
46
47 number of individuals in each group. The “ ≥ 35 years” group was for this estimation defined as ages
48
49 between 35 to 65. The smallpox vaccination coverage estimation for the age group ≥ 35 in 2016 was
50
51 changed to ≥ 45 to ensure a steady smallpox vaccination coverage.
52
53
54
55
56

57 A small validation study based on a city register of smallpox vaccination from Bissau showed a
58
59 sensitivity of 90% (95% CI, 80-95%) by using smallpox scars as proxies for registered smallpox
60

1
2 vaccinations (62 individuals had smallpox scars in community surveys out of 69 registered as
3
4 smallpox vaccinated in the city register).(7)
5

6 **Estimates of female/male HIV-1 prevalence ratios**

7
8
9 Three HIV-1 prevalence surveys were carried out in parallel both in Bissau (1987, 1996, 2006)(4)
10
11 and Caió (1990, 1997, 2007).(5) An additional survey was carried out in Bissau in 2016.(9) In these
12
13 surveys, all individuals aged 15 years or older from randomly selected households were interviewed
14
15 and tested for HIV provided consent.
16
17

18
19
20 The HIV-1 infection data were reported by sex and 10-year age groups from 15 years of age. Based
21
22 on these data, we constructed a dataset with the number of observed individuals by sex, age group
23
24 [15-34; ≥ 35] and HIV-1 status for each of the HIV surveys. The reason for the age cut-off of 35
25
26 years was that the last smallpox vaccination campaign in Guinea-Bissau was in 1975 and pre-school
27
28 children were rarely vaccinated (7) resulting in a decreasing smallpox vaccination among 15-34-
29
30 year-old individuals across HIV survey years. The combined estimates for 2016 were based on
31
32 Bissau, as no HIV survey had been carried out in Caió; in this survey, the age range was changed to
33
34 ≥ 45 to ensure a steady smallpox vaccination coverage.
35
36
37
38
39

40
41 The female/male HIV-1 prevalence ratio in two specific age groups was of interest in itself, but
42
43 also, by using such as comparison, we could to some extent disregard time trends in the general
44
45 spread of HIV-1 worldwide and in the focus on prophylaxis and treatment, which would affect both
46
47 sexes and all age groups.
48
49
50

51 **Statistical analysis**

52
53 We used R 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria) to estimate the
54
55 female/male HIV-1 prevalence ratios among individuals 15-34 years and ≥ 35 years for each HIV
56
57 survey (confidence intervals were calculated using the “epitools” package for risk ratios). Individual
58
59
60

1
2 level data sets were reconstructed for the surveys based on the summary tables in (4, 5, 9). To
3
4 estimate the probability of data showing the observed trend in female/male HIV-1 prevalence by the
5
6 combined HIV survey years (1987-90, 1996-97, 2006-07) by chance, we fitted a logistic regression
7
8 on HIV-1 status depending on HIV survey year as a linear and quadratic effect, sex and the
9
10 interaction between a linear effect of HIV survey year and sex. The model was fitted separately for
11
12 the individuals aged 15-34 and ≥ 35 . We interpreted the p-value for the interaction as a test for a
13
14 homogeneous association between sex and a linear change in HIV-1 prevalence across survey years.
15
16
17
18
19

20 21 Results

22
23
24 For the age group ≥ 35 years (≥ 45 years in 2016), the estimated smallpox vaccination coverage
25
26 was similar across all the HIV surveys (fluctuating between 66% and 77%, Figure 1). As expected,
27
28 the smallpox vaccination coverage decreased over HIV survey years for the age group 15-34 years
29
30 (from 62% in 1987 to 0% in 2016, Figure 1). There was no indication that the smallpox vaccination
31
32 coverage differed between women and men (Supplementary figures 1 and 2). The general
33
34 prevalence of HIV-1 among adults ≥ 15 years of age increased from 0% (0/649) in 1987 to 4.6%
35
36 (118/2548) in 2006 in Bissau and from 0.5% (14/2770) in 1990 to 3.6% (105/2895) in 2007 in Caió.
37
38 In 2016 in Bissau, the HIV-1 prevalence among adults over 15 was 4.0% (104/2601).
39
40
41
42
43
44

45 As seen in Table 1, there was an increase in the female/male HIV-1 prevalence ratio among
46
47 individuals 15-34 years from the earliest to the latest conducted HIV surveys, the pattern being
48
49 similar in Bissau and Caió. Combined, the female prevalence increased from 0.3% to 4.3% from
50
51 1987-1990 to 2006-07, whereas the male prevalence increased from 0.3% to 1.9% in the same
52
53 period. The female/male HIV-1 prevalence ratio was 1.00 (95% confidence interval (CI) 0.17-5.99)
54
55 in 1987-90, 1.16 (95% CI 0.69-1.93) in 1996-97 and 2.32 (95% CI 1.51-3.56) in 2006-07. In a
56
57 logistic regression, the interaction-test for a homogeneous association between sex and a linear
58
59
60

1
2 change in HIV-1 prevalence across survey years for the individuals aged 15-34 years gave a p-value
3
4 of 0.04. Latest in Bissau in 2016, the female/male HIV-1 prevalence ratio was further increased to
5
6 5.41 (95% CI 2.15-13.61).
7
8
9

10
11 The older age group with steady smallpox vaccination coverage had no increase in the female/male
12
13 HIV-1 prevalence ratio. Combined, the female prevalence increased from 0.7% to 5.0% from 1987-
14
15 1990 to 2006-07, whereas the male prevalence increased from 0.4% to 6.2% in the same period.
16
17 Thus, the female/male HIV-1 prevalence ratios were 1.93 (95% CI 0.40-9.25) in 1987-90, 1.32
18
19 (95% CI 0.83-2.10) in 1996-97, 0.81 (95% CI 0.56-1.16) in 2006-07. The test of interaction for a
20
21 homogeneous association between sex and a linear change in HIV-1 prevalence across surveys gave
22
23 a p-value of 0.07 and the direction trended towards the opposite direction than for the younger age
24
25 group. The female/male HIV-1 prevalence ratio was 1.03 (95% CI 0.47-2.25) in 2016 in Bissau.
26
27
28
29
30

31
32 The combined female/male HIV-1 prevalence ratios are illustrated in Figure 2. Relative to the F/M
33
34 prevalence ratio among the older age group, the F/M prevalence ratio in the 15-34 years age group
35
36 increased from 0.52 (95% CI 0.05-5.61) in 1987-90 to 0.88 (95% CI 0.44-1.75) in 1996-97 to 2.88
37
38 (95% CI 1.64-5.05) in 2006-07 to 5.26 (95% CI 1.57-17.65) in 2016 (2016 estimates were only
39
40 based on Bissau data) (ratios of ratios based on Table 1).
41
42
43
44
45

46 Discussion

47
48

49
50 As we had hypothesized the female/male HIV-1 prevalence ratio increased for the age group 15-34
51
52 years, as the proportion with smallpox vaccination scars decreased, whereas the female/male HIV-1
53
54 prevalence ratio remained unchanged for the age group ≥ 35 years, which had a steady smallpox
55
56 vaccination coverage over the HIV-1 survey years.
57
58
59
60

Strengths and limitations

This study was based on information from large HIV surveys carried out over 20-30 years in two different settings, urban and rural Guinea-Bissau. As no central smallpox vaccination register exists in Guinea-Bissau, we used smallpox vaccination scars as a proxy for the smallpox vaccination coverage. Some BCG vaccination scars and accidental wounds may have been misclassified as smallpox vaccination scars, but misclassification is unlikely to be sex-differential. Participation in the HIV surveys varied only slightly across the survey years in Bissau, being 86% in 1987, 85% in 1996, 79% in 2006 and 83% in 2016; furthermore, the HIV prevalence in participants, who were easy to reach, was similar to the prevalence in those who were difficult to reach.^(4, 9) Hence, differential participation in different study years is unlikely to be a confounding factor.

The ecological design enabled us to investigate a potentially important hypothesis, but the results needs to be interpreted with caution since this design can be vulnerable to misinterpretations. By using the ratio of HIV-1 prevalence between sexes and within age groups, we could to some extent disregard time trends such as the general spread of HIV-1 worldwide and the roll out of prophylaxis and treatment affecting both sexes and all age groups.

Female/male HIV-1 prevalence trends in Sub-Saharan Africa

Consistent with our finding, cross sectional surveys from Malawi,⁽¹⁰⁾ Zambia,⁽¹¹⁾ and South Africa⁽¹²⁾ show that the birth cohorts who are too young to have been smallpox vaccinated have an increased female/male HIV prevalence compared with older birth cohorts, who are likely to have been smallpox vaccinated before the worldwide phase-out in 1980.

Furthermore, UNAIDS data for the female/male HIV-1 prevalence from 1985 to 2003 in Sub-Saharan Africa shows that in the 15-49-year-old age group, the number of HIV-1 affected women

1
2 began to increase over the number of men during the early 1990s.(13) The female/male HIV-1 ratio
3
4 increased at the same time as the smallpox vaccination coverage decreased after 1980. A multi-
5
6 country study using repeated national representative demographic and health surveys on HIV
7
8 prevalence in Sub-Saharan Africa during the 2000s did not find an increasing female/male HIV
9
10 prevalence ratio.(14) In contrast to the HIV surveys from Guinea-Bissau, where there was a clear
11
12 increase in the prevalence of HIV-1, the reported HIV prevalence generally decreased between
13
14 repeated surveys in other regions of Africa.(14) The female/male HIV prevalence ratio may be
15
16 influenced by multiple factors, and the introduction of HIV treatment, which only took place in the
17
18 late 2000s in Guinea-Bissau,(15) may have blurred the female/male HIV prevalence ratio trends in
19
20 the 2000s surveys from Sub-Saharan Africa.
21
22
23
24
25
26

27 **Potential causes of sex differences in HIV-1**

28
29 The sex differences in the susceptibility to HIV-1 could theoretically be due to physiological,
30
31 hormonal or local microbial differences, and higher prevalence of sexually transmitted diseases
32
33 causing a higher male-to-female than female-to-male HIV transmission rate.(13) These
34
35 explanations would however not explain why young women in Sub-Saharan Africa did not have a
36
37 higher HIV-1 prevalence than men when the HIV epidemic started. Our results showed that women
38
39 and men in the age group <35 years had similar HIV-1 prevalence in 1987-1990 of 0.3% but while
40
41 men's prevalence did not increase much in the younger age group, potentially due to more focus on
42
43 availability and use of condoms over time, women's prevalence continued to increase (Table 1). It
44
45 may be that female's increased susceptibility were neutralized by the smallpox vaccination and
46
47 became expressed when smallpox vaccination was stopped.
48
49
50
51
52
53
54

55 Alternative explanations for the sex-age-time pattern may be sought in social and cultural changes
56
57 over time, including gender-power imbalances.(13) Analyses of sexual mixing patterns from South
58
59 Africa(12) suggest that since young women often have sexual relations with older men, then as the
60

1 prevalence of HIV-1 increases among older men the prevalence among young women will follow.
2
3
4 We have no specific data to assess possible changes over time in the frequency of sexual relations
5
6 across age groups. All ethnic groups in Guinea-Bissau has a taboo on intercourse while the mother
7
8 is breastfeeding for 1½-3 years, which may have created a permissive attitude towards extra-marital
9
10 sexual relationships (possible causing sexual relations between older men and younger women). We
11
12 have documented such taboo on intercourse while breastfeeding and a permissive attitude back to
13
14 the 1980s in Guinea-Bissau (16) so it is clearly not a new phenomenon. While it is possible that
15
16 behavioral patterns became increasingly permissive of extra-marital sexual relationships in a setting
17
18 with rapidly increasing urbanization, it seems unlikely that the same change would have happened
19
20 in a rural setting. We find it unlikely that the similar pattern of increasing female/male HIV-1
21
22 prevalence ratio in both an urban and a rural setting can be explained merely by changes in sexual
23
24 behavior patterns. During the study period, there may have been increased awareness and
25
26 availability of condoms, but this would likely have affected the risk of acquiring HIV equally in
27
28 both sexes or if anything diminished the risk in females relative to males.
29
30
31
32
33
34
35

36 It should be noted, though, that the observation that the female/male HIV-1 prevalence ratio for the
37
38 age group 15-34 seems to continue to increase in Bissau with a ratio of 5.41 (95% CI 2.15-13.61) in
39
40 2016 compared with 2.34 (95% CI 1.35-4.04) in 2006, despite the prevalence of smallpox
41
42 vaccination coverage for this age group is likely to only have changed from 4% to 0%, suggests that
43
44 other factors continue to affect the susceptibility of HIV-1 differently for men and women.
45
46
47
48
49

50 **Biological mechanisms**

51
52 The CCR5 is fundamental for establishing HIV-1 infection.(17) The CCR5-delta-32 deletion
53
54 confers resistance to HIV-1 by preventing expression of the CCR5 receptor; this allele provides
55
56 almost complete resistance to HIV-1 in homozygous individuals.(17) A recent immunological study
57
58 found that cells from smallpox vaccinated individuals had up to 5-fold reduction in CCR-5 tropic
59
60

1
2 HIV-1 replication *in vitro*,⁽²⁾ which supports a role for smallpox vaccine in HIV-1 prevention
3
4 through heterologous immunity. A recent study did not show an association between smallpox
5
6 vaccination scar and CCR5 expression on the surface of peripheral T-lymphocytes among HIV
7
8 seronegative women old enough to have had a chance of being smallpox vaccinated;⁽¹⁸⁾ this may
9
10 be due to delay between smallpox vaccination and immunological testing of more than four decades
11
12 or that the smallpox-unvaccinated control group had received another immunomodulator, the BCG
13
14 vaccine.⁽¹⁹⁾
15
16
17
18
19

20 In animal models, administrating smallpox vaccination via skin scarification increased immune
21
22 response and survival compared with other modes of administration.⁽²⁰⁾ Murine studies have
23
24 shown that intradermal smallpox vaccination induced long-lived non-recirculating CD8⁺ skin
25
26 resident T-memory cells that resided within the entire skin and protected against reinfection.⁽²¹⁾
27
28 This indicates that vaccination can spread throughout the entire epithelial surface to create a
29
30 “shield” against infection.
31
32
33
34
35

36 Smallpox vaccine may also affect the innate immune system more broadly; in a very recent study,
37
38 human monocytes trained with smallpox vaccine showed significantly increased IL-6 and TNF- α
39
40 production to stimulation with non-related stimuli, compared to non-trained monocytes.⁽²²⁾
41
42
43
44

45 Overall, there is some immunological evidence to support that smallpox vaccination can provide
46
47 cross-protection against HIV-1 infection. None of the above studies reported effects by sex, but it is
48
49 plausible that an epithelial protection might be particularly protective against vaginally acquired
50
51 HIV-1 infection.
52
53
54
55
56
57
58
59
60

Conclusion

Our hypothesis that termination of smallpox vaccination may have increased the female/male HIV-1 prevalence ratio was compatible with the results from ecological studies in Guinea-Bissau. More research is needed to test this hypothesis, and we hope other research groups will test it in individual-based data. If the hypothesis is true, studies of smallpox vaccination could inform HIV-1 vaccine research.

Author contact information:

Andreas Rieckmann, Research Center for Vitamins and Vaccines (CVIVA), Bandim Health Project, Statens Serum Institut, Artillerivej 5, DK-2300 Copenhagen S, Denmark and Section of Epidemiology, Department of Public Health, University of Copenhagen, Copenhagen, Denmark.

anri@ssi.dk

Marie Villumsen, Center for Clinical Research and Prevention, Bispebjerg and Frederiksberg Hospital, Capital Region, Copenhagen, Denmark. mvm.villumsen@gmail.com

Bo Langhoff Hønge, Department of Clinical Immunology, Aarhus University Hospital, Denmark and Bandim Health Project, Indepth Network, Apartado 861, Bissau, Guinea-Bissau.

bohonge@gmail.com

Signe Sørup, Research Center for Vitamins and Vaccines (CVIVA), Bandim Health Project, Statens Serum Institut, Artillerivej 5, DK-2300 Copenhagen S, Denmark and Department of Clinical Epidemiology, Aarhus University, Olof Palmes Allé 43-45, DK-8200 Aarhus N, Denmark.

SGS@ssi.dk

Amabélia Rodrigues, Bandim Health Project, Indepth Network, Apartado 861, Bissau, Guinea-Bissau. a.rodrigues@bandim.org

Zacarias José da Silva, National Institute of Public Health (INASA), CP 1013, Bissau, Guinea-Bissau. zacarias55@hotmail.com

1
2 Hilton Whittle, London School of Hygiene and Tropical Medicine, Keppel Street, London, UK,
3
4 hcwhittle@yahoo.co.uk
5

6 Christine Stabell Benn, Research Center for Vitamins and Vaccines (CVIVA), Bandim Health
7
8 Project, Statens Serum Institut, Artillerivej 5, DK-2300 Copenhagen S, Denmark and OPEN,
9
10 Odense University Hospital/Institute of Clinical Research, University of Southern Denmark,
11
12 Odense, Denmark. CB@ssi.dk
13
14

15 Peter Aaby, Bandim Health Project, Indepth Network, Apartado 861, Bissau, Guinea-Bissau.
16
17 p.aaby@bandim.org
18
19
20
21
22
23
24
25

26 **Author Statement:** All authors have made substantial contributions to this work. AR and PA
27
28 drafted the manuscript, which has been revised critically by all authors.
29

30 **Data Statement:** The Bandim Health Project (bandim@ssi.dk) can be contacted for data requests.
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

References

1. Mayr A. Taking advantage of the positive side-effects of smallpox vaccination. *Journal of veterinary medicine B, Infectious diseases and veterinary public health*. 2004;51(5):199-201.
2. Weinstein RS, Weinstein MM, Alibek K, Bukrinsky MI, Brichacek B. Significantly reduced CCR5-tropic HIV-1 replication in vitro in cells from subjects previously immunized with Vaccinia Virus. *BMC immunology*. 2010;11:23.
3. Rieckmann A, Villumsen M, Jensen ML, Ravn H, Silva ZJd, Sørup S, et al. The effect of smallpox and BCG vaccination on the risk of HIV-1 infection in Guinea-Bissau and Denmark. *Open Forum Infect Dis*. 2017.
4. da Silva ZJ, Oliveira I, Andersen A, Dias F, Rodrigues A, Holmgren B, et al. Changes in prevalence and incidence of HIV-1, HIV-2 and dual infections in urban areas of Bissau, Guinea-Bissau: is HIV-2 disappearing? *Aids*. 2008;22(10):1195-202.
5. Tienen C, van der Loeff MS, Zaman SM, Vincent T, Sarge-Njie R, Peterson I, et al. Two distinct epidemics: the rise of HIV-1 and decline of HIV-2 infection between 1990 and 2007 in rural Guinea-Bissau. *Journal of acquired immune deficiency syndromes*. 2010;53(5):640-7.
6. Rieckmann A, Villumsen M, Jensen ML, Ravn H, da Silva ZJ, Sorup S, et al. The Effect of Smallpox and Bacillus Calmette-Guerin Vaccination on the Risk of Human Immunodeficiency Virus-1 Infection in Guinea-Bissau and Denmark. *Open forum infectious diseases*. 2017;4(3):ofx130.
7. Aaby P, Gustafson P, Roth A, Rodrigues A, Fernandes M, Sodemann M, et al. Vaccinia scars associated with better survival for adults. An observational study from Guinea-Bissau. *Vaccine*. 2006;24(29-30):5718-25.
8. Jensen ML, Dave S, Schim van der Loeff M, da Costa C, Vincent T, Leligdowicz A, et al. Vaccinia scars associated with improved survival among adults in rural Guinea-Bissau. *PloS one*. 2006;1:e101.
9. Olesen JS, Jespersen S, da Silva ZJ, Rodrigues A, Erikstrup C, Aaby P, et al. HIV-2 continues to decrease, whereas HIV-1 is stabilizing in Guinea-Bissau. *Aids*. 2018;32(9):1193-8.
10. Maman D, Chilima B, Masiku C, Ayouba A, Masson S, Szumilin E, et al. Closer to 90-90-90. The cascade of care after 10 years of ART scale-up in rural Malawi: a population study. *Journal of the International AIDS Society*. 2016;19(1):20673.
11. Chanda-Kapata P, Kapata N, Klinkenberg E, William N, Mazyanga L, Musukwa K, et al. The adult prevalence of HIV in Zambia: results from a population based mobile testing survey conducted in 2013-2014. *AIDS research and therapy*. 2016;13:4.
12. de Oliveira T, Kharsany AB, Graf T, Cawood C, Khanyile D, Grobler A, et al. Transmission networks and risk of HIV infection in KwaZulu-Natal, South Africa: a community-wide phylogenetic study. *The lancet HIV*. 2017;4(1):e41-e50.
13. Quinn TC, Overbaugh J. HIV/AIDS in women: an expanding epidemic. *Science*. 2005;308(5728):1582-3.
14. Hegdahl HK, Fylkesnes KM, Sandoy IF. Sex differences in HIV prevalence persist over time: Evidence from 18 countries in Sub-Saharan Africa. *PloS one*. 2016;11(2):e0148502.
15. Jespersen S, Honge BL, Oliveira I, Medina C, da Silva Te D, Correia FG, et al. Challenges facing HIV treatment in Guinea-Bissau: the benefits of international research collaborations. *Bulletin of the World Health Organization*. 2014;92(12):909-14.
16. Høgsborg M, Aaby P. Sexual relations, use of condoms and perceptions of AIDS in an urban area of Guinea-Bissau with a high prevalence of HIV-2. *Sexual behaviour and networking: anthropological and socio-cultural studies on the transmission of HIV*, edited by Tim Dyson Liege, Belgium, Editions Derouaux-Ordina, [1992] 203-31. 1992.
17. Dean M, Carrington M, Winkler C, Huttley GA, Smith MW, Allikmets R, et al. Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. *Hemophilia Growth and Development Study, Multicenter AIDS Cohort Study, Multicenter Hemophilia Cohort Study, San Francisco City Cohort, ALIVE Study*. *Science*. 1996;273(5283):1856-62.
18. Beck KB, Honge BL, Olesen JS, Petersen MS, Jespersen S, Wejse C, et al. Long-term effects of smallpox vaccination on expression of the HIV-1 co-receptor CCR5 in women. *PloS one*. 2018;13(11):e0207259.
19. Netea MG, Joosten LA, Latz E, Mills KH, Natoli G, Stunnenberg HG, et al. Trained immunity: A program of innate immune memory in health and disease. *Science*. 2016;352(6284):aaf1098.
20. Rice AD, Adams MM, Lindsey SF, Swetnam DM, Manning BR, Smith AJ, et al. Protective properties of vaccinia virus-based vaccines: skin scarification promotes a nonspecific immune response that protects against orthopoxvirus disease. *Journal of virology*. 2014;88(14):7753-63.
21. Jiang X, Clark RA, Liu L, Wagers AJ, Fuhlbrigge RC, Kupper TS. Skin infection generates non-migratory memory CD8+ T(RM) cells providing global skin immunity. *Nature*. 2012;483(7388):227-31.
22. Blok BA, Jensen KJ, Aaby P, Fomsgaard A, van Crevel R, Benn CS, et al. Opposite effects of Vaccinia and modified Vaccinia Ankara on trained immunity. *European journal of clinical microbiology & infectious diseases* : official publication of the European Society of Clinical Microbiology. 2019;38(3):449-56.

1
2 **Figure 1. Estimated smallpox vaccination scar prevalence in different age groups for each**
3
4 **sequential HIV survey**
5

6
7
8 Insert figure 1
9

10 Based on data from Bissau, Guinea-Bissau, previously published.(6) The estimation for the age
11 group ≥ 35 in 2016 was changed to ≥ 45 to ensure a steady smallpox vaccination coverage.
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46

1 **Table 1. The female/male HIV-1 prevalence ratio (PR) by age group, survey year and study site**

Age group	15-34 years (decreasing smallpox vaccination rates in later years; see Figure 1)				≥35 years (steady smallpox vaccination rate over time; see Figure 1)			
	Estimated smallpox coverage	HIV-1 prevalence (%)		Female/male PR (95% CI)	Estimated smallpox coverage	HIV-1 prevalence (%)		Female/male PR (95% CI)
Study site and survey year		Female	Male			Female	Male	
Caió								
1990	62%	0.3% (3/861)	0.4% (2/541)	0.94 (0.16-5.62)	72%	0.8% (7/907)	0.4% (2/461)	1.78 (0.37-8.53)
1997	27%	1.8% (17/958)	1.9% (14/738)	0.94 (0.46-1.89)	77%	4.4% (41/943)	2.8% (13/471)	1.58 (0.85-2.91)
2007	4%	3.2% (28/885)	1.5% (11/742)	2.13 (1.07-4.26)	71%	4.8% (41/850)	6.0% (25/418)	0.81 (0.50-1.31)
Bissau								
1987	62%	0% (0/243)	0% (0/197)	NA	72%	0% (0/110)	0% (0/99)	NA
1996	27%	2.2% (19/881)	1.5% (10/680)	1.47 (0.69-3.13)	77%	3.3% (13/394)	3.5% (12/346)	0.95 (0.44-2.06)
2006	4%	5.3% (56/1056)	2.3% (16/705)	2.34 (1.35-4.04)	71%	5.4% (25/466)	6.5% (21/321)	0.82 (0.47-1.44)
2016	0%	4.2% (41/983)	0.8% (5/648)	5.41 (2.15-13.61)	66%	5.2% (13/252)**	5.0% (11/219)**	1.03 (0.48-2.25)*
Combined								
1987-90	62%	0.3% (3/1104)	0.3% (2/738)	1.00 (0.17-5.99)	72%	0.7% (7/1017)	0.4% (2/560)	1.93 (0.40-9.25)
1996-97	27%	2.0% (36/1839)	1.7% (24/1418)	1.16 (0.69-1.93)	77%	4.0% (54/1337)	3.1% (25/817)	1.32 (0.83-2.10)

2006-07	4%	4.3% (84/1941)	1.9% (27/1447)	2.32 (1.51-3.56)	71%	5.0% (66/1316)	6.2% (46/739)	0.81 (0.56-1.16)
---------	----	-------------------	-------------------	------------------	-----	-------------------	------------------	------------------

2 Data are extracted from (4, 5, 9).

3 * This estimate is only based on information from Bissau.

4 ** The estimation for the age group ≥ 35 in 2016 was changed to ≥ 45 to ensure a steady smallpox vaccination coverage.

For peer review only

1
2
3 **Figure 2. Female/male HIV-1 prevalence ratios, Bissau and Caió**
4

5
6 Insert figure 2
7

8
9 Visualisations of estimates from Table 1. Circles and triangles represent point estimates and lines
10 represent the 95% confidence intervals. The estimation for the age group ≥ 35 in 2016 was only
11 from Bissau and was changed to ≥ 45 to ensure a steady smallpox vaccination coverage.
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

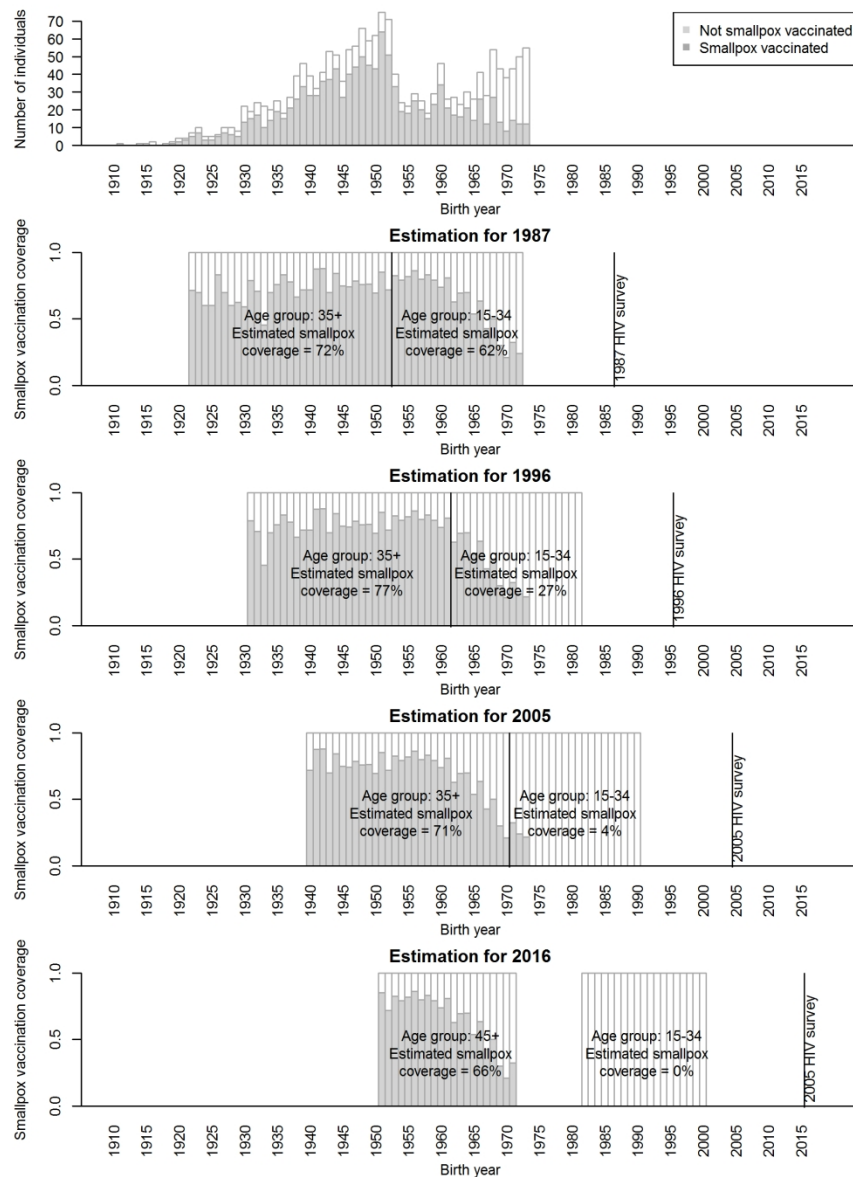


Figure 1. Estimated smallpox vaccination scar prevalence in different age groups for each sequential HIV survey.

Based on data from Bissau, Guinea-Bissau, previously published.(6) The estimation for the age group ≥ 35 in 2016 was changed to ≥ 45 to ensure a steady smallpox vaccination coverage

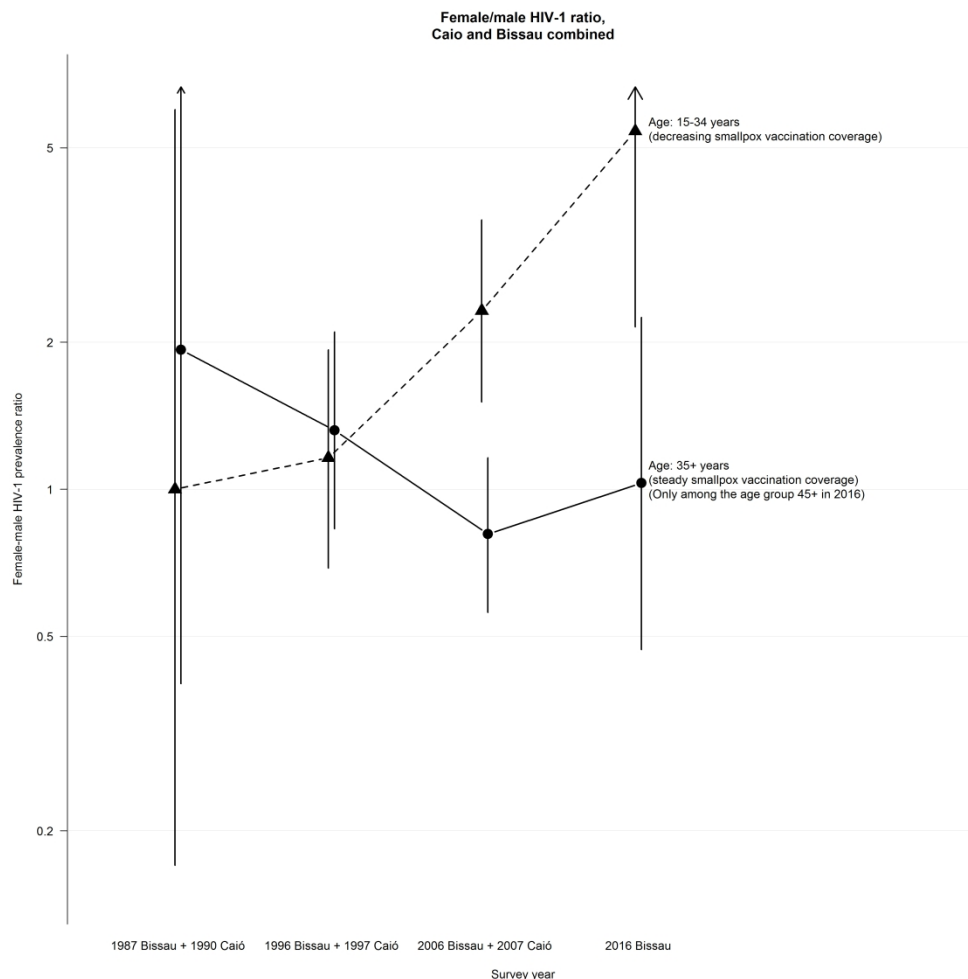
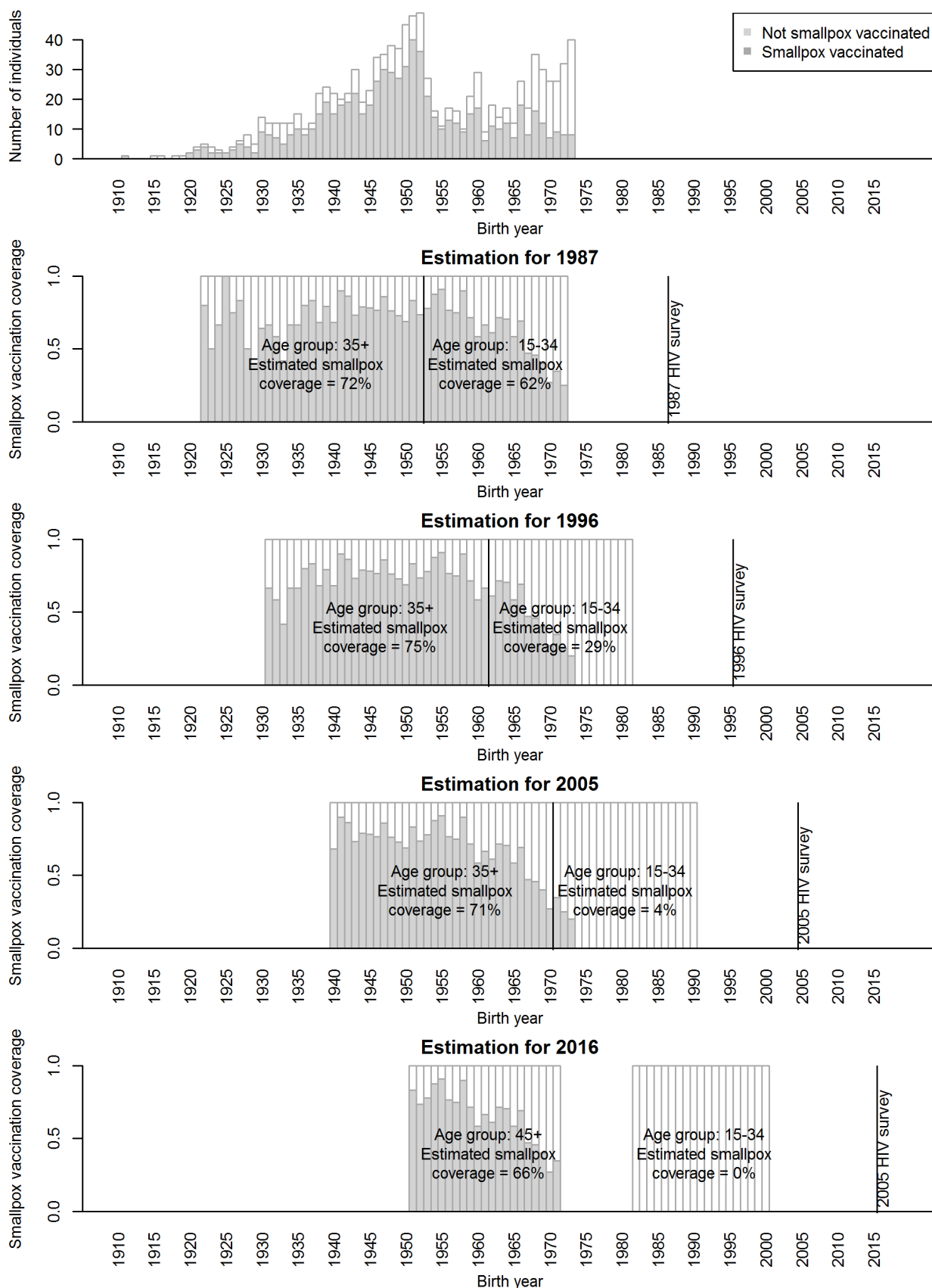


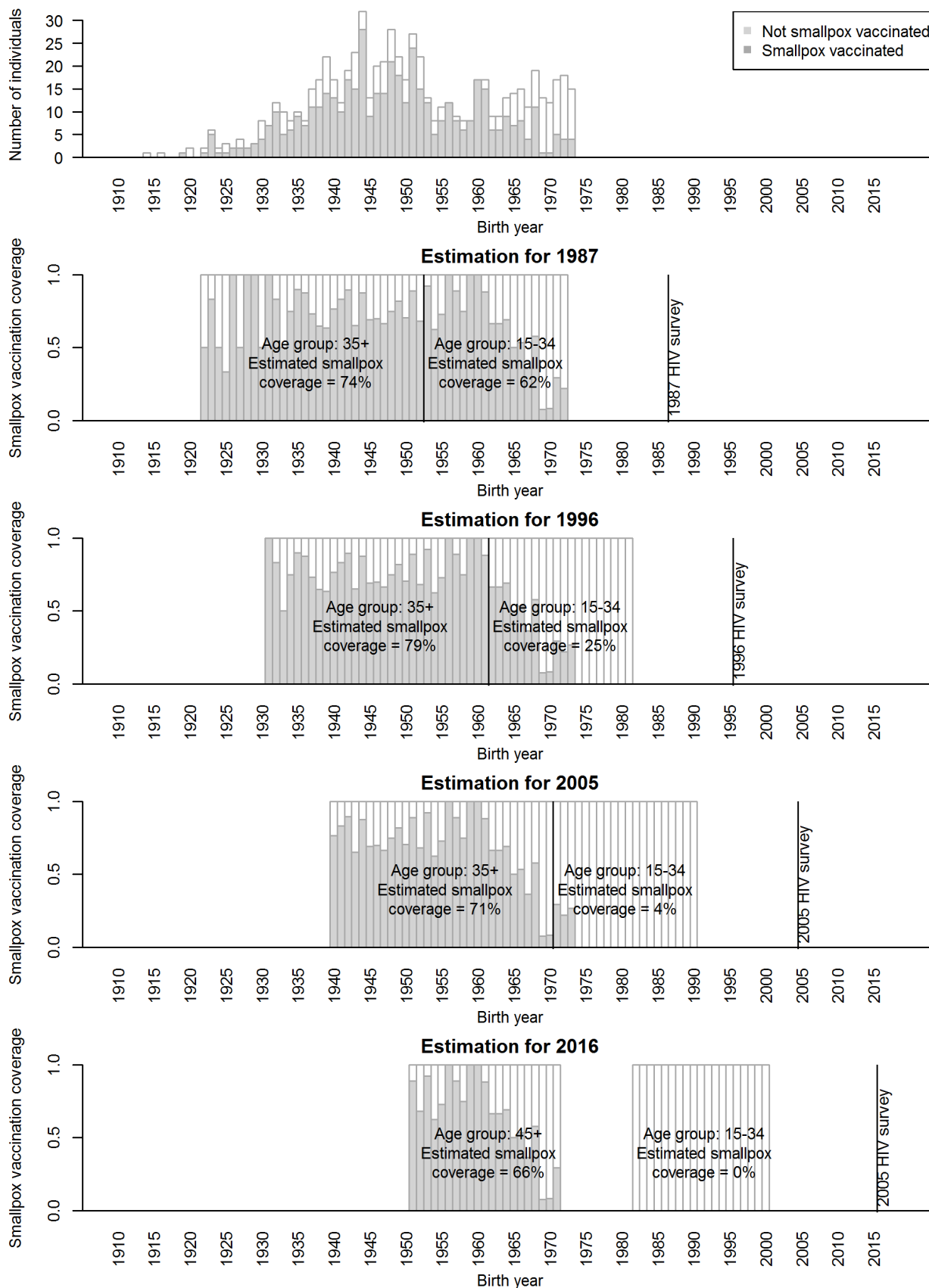
Figure 2. Female/male HIV-1 prevalence ratios, Bissau and Caió.

Visualisations of estimates from Table 1. Circles and triangles represent point estimates and lines represent the 95% confidence intervals. The estimation for the age group ≥ 35 in 2016 was only from Bissau and was changed to ≥ 45 to ensure a steady smallpox vaccination coverage.

Supplementary figure 1. Estimated smallpox vaccination scar prevalence in different age groups for each sequential HIV survey among women



Supplementary figure 2. Estimated smallpox vaccination scar prevalence in different age groups for each sequential HIV survey among men



BMJ Open

The termination of smallpox vaccination may have increased the female/male HIV-1 prevalence ratio: an ecological study from Guinea-Bissau testing a hypothesis

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2019-031415.R1
Article Type:	Original research
Date Submitted by the Author:	01-Aug-2019
Complete List of Authors:	Rieckmann, Andreas; Bandim Health Project, Statens Serum Institut Villumsen, Marie Hønge, Bo; Aarhus University Hospital, Department of Infectious Diseases Sørup, Signe Rodrigues, Amabelia; Bandim Health Project, Bandim Health Project, da Silva, Zacarias Whittle, Hilton; London School of Hygiene and Tropical Medicine, Benn, Christine; Statens Serum Institut, Bandim Health Project; University of Southern Denmark, OPEN Aaby, Peter; Bandim Health Project,
Primary Subject Heading:	HIV/AIDS
Secondary Subject Heading:	Public health
Keywords:	Heterologous immunity, HIV-1, Non-specific effects of vaccines, Smallpox vaccination, Vaccinia

SCHOLARONE™
Manuscripts

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

The termination of smallpox vaccination may have increased the female/male HIV-1 prevalence ratio: an ecological study from Guinea-Bissau testing a hypothesis

Andreas Rieckmann^{1,2,3} *, Marie Villumsen⁴, Bo Langhoff Hønge^{5,6}, Signe Sørup^{1,2,7}, Amabélia Rodrigues⁶, Zacarias José da Silva⁸, Hilton Whittle⁹, Christine Stabell Benn^{1,2}, Peter Aaby⁶

¹ Bandim Health Project, OPEN, Institute of Clinical Research, University of Southern Denmark/Odense University Hospital, Odense, Denmark.

² Research Center for Vitamins and Vaccines (CVIVA), Bandim Health Project, Statens Serum Institut, Artillerivej 5, DK-2300 Copenhagen S, Denmark

³ Section of Epidemiology, Department of Public Health, University of Copenhagen, Copenhagen, Denmark

⁴ Center for Clinical Research and Prevention, Bispebjerg and Frederiksberg Hospital, Capital Region, Copenhagen, Denmark

⁵ Department of Clinical Immunology, Aarhus University Hospital, Denmark

⁶ Bandim Health Project, Indepth Network, Apartado 861, Bissau, Guinea-Bissau

⁷ Department of Clinical Epidemiology, Aarhus University, Olof Palmes Allé 43-45, DK-8200 Aarhus N, Denmark

⁸ National Institute of Public Health (INASA), CP 1013, Bissau, Guinea-Bissau

⁹ London School of Hygiene and Tropical Medicine, Keppel Street, London, UK

* Corresponding author: Andreas Rieckmann, anri@ssi.dk

Word count: Abstract: 298, Manuscript 2942, References 23

Tables: 1, Figures: 2, Supplementary figures 2.

1
2 **Financial support:** The Danish National Research Foundation (DNRF) supported the establishment
3
4 of Research Center for Vitamins and Vaccines [DNRF108]. AR was supported by an unrestricted
5
6 Faculty of Health Sciences-scholarship from University of Southern Denmark. SS was supported by
7
8 a grant from the Danish Council for Independent Research [DFR – 4183-00316].
9

10
11 **Disclosures:** The authors have no conflicts of interest.
12

13 **Ethics statement:** Our study is based on published results from 3 original research papers. The
14
15 study by da Silva et al. was approved by the Guinea-Bissau Government Ethics Committee and the
16
17 Danish Central Scientific Ethics Committee. The study by van Tienen was approved by the Gambia
18
19 Government/MRC Laboratories Joint Ethics Committee and by the Ministry of Health of Guinea-
20
21 Bissau. The study by Olesen et al. was approved by the National Research Ethics Committee in
22
23 Guinea-Bissau and received consultative approval from the National Research Ethics Committee of
24
25 Denmark.
26
27

28
29 **Data access:** Information about HIV-1 was extracted from published results from original research
30
31 papers carried out in parallel both in Bissau (1987, 1996, 2006 [da Silva et al], 2016 [Olesen et al.])
32
33 and Caió (1990, 1997, 2007 [van Tienen et al.] in Guinea-Bissau. Information about smallpox
34
35 vaccination was based on data from a cohort of individuals, who had both participated in a smallpox
36
37 vaccination scar survey (2005-2007) and an HIV prevalence survey (2004-2006) conducted in
38
39 Bissau. The Bandim Health Project (bandim@ssi.dk) can be contacted for data requests.
40
41
42
43
44

45 **Author's contributions :** AnR, MV, BLH, SS, AmR, ZJS, HW, CSB, and PA made substantial
46
47 contributions to this work. AnR and PA drafted the manuscript, which has been revised critically by
48
49 MV, BLH, SS, AmR, ZJS, HW, and CSB.
50
51
52
53
54
55
56
57
58
59
60

Abstract

Objective: In Guinea-Bissau, West Africa, we observed that having a smallpox vaccination scar vs. not having it was associated with lower HIV-1 prevalence, more strongly for women. If this represents a causal effect, the female/male HIV-1 prevalence ratio would increase for birth cohorts no longer receiving smallpox vaccination due to the termination of this vaccine.

Design: An ecological design using HIV surveys and information about smallpox vaccination coverage.

Setting: Urban and rural Guinea-Bissau.

Participants: Participants in HIV surveys were grouped into an age group with decreasing smallpox vaccination coverage (15-34 years) and an age group with steady smallpox vaccination coverage (≥ 35 years).

Interventions: The cessation of smallpox vaccination

Primary and secondary outcome measures: HIV-1 prevalence

Results: At both study sites, the female/male HIV-1 prevalence ratio increased over time for the age group with decreasing smallpox vaccination coverage; the combined female/male HIV-1 prevalence ratio among 15-34-year-olds was 1.00 (95% confidence interval (CI) 0.17-5.99) in 1987-90, 1.16 (95% CI 0.69-1.93) in 1996-97, 2.32 (95% CI 1.51-3.56) in 2006-07 (p-value for no linear trend=0.04). There was no increase in the female-male HIV-1 prevalence ratio for the age group ≥ 35 years with steady smallpox vaccination coverage (1.93 (95% CI 0.40-9.25) in 1987-90, 1.32 (95% CI 0.83-2.10) in 1996-97, 0.81 (95% CI 0.56-1.16) in 2006-07 (p-value for no linear trend=0.07)).

Conclusions: Thus, data was compatible with the deduction that terminating smallpox vaccination may have increased the susceptibility to HIV-1 relatively more for women than for men. Hence, terminating smallpox vaccination may have contributed to the striking global increase in the female/male HIV-1 prevalence ratio among young individuals. Due to the potential fallacies of ecological studies, the results should be interpreted carefully, and more research is needed to test

1
2 this hypothesis. If the hypothesis is true, studies of smallpox vaccination could inform HIV-1
3
4 vaccine research.
5
6
7

8
9 Key words: Heterologous immunity; HIV-1; Non-specific effects of vaccines; Smallpox
10
11 vaccination; Vaccinia.
12
13
14
15
16
17

18 Article summary

19 Strengths and limitations of this study

- 22 • The ecological design allowed us to assess a deduction of the hypothesis: Smallpox vaccination has a
23 protective effect against HIV-1, which is stronger in women than men.
- 24 • To increase the robustness of the results, we utilized parallel data from both urban and rural Guinea-Bissau.
- 25 • Ecological studies should be interpreted carefully as spurious associations can arise, and thus triangulation
26 with existing studies are necessary.
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Introduction

Vaccination against smallpox infections was stopped globally in 1980 following the eradication of smallpox in 1977. It has been reported that smallpox vaccination reduced susceptibility to unrelated infectious diseases,(1) and in immunological *in vitro* studies, smallpox vaccination was associated with an up to 5-fold reduction in C-C chemokine receptor 5 (CCR5) tropic HIV-1 replication.(2) Based on vaccination scar readings in Guinea-Bissau and school health records in Denmark, we have shown that smallpox vaccination (and Bacille Calmette-Guérin vaccination [BCG]) was associated with a lower risk of HIV-1.(3) The adjusted odds ratio for HIV-1 infection was 0.52 (95% CI 0.32-0.84) for women and 0.77 (95% CI 0.48-1.24) for men. This association was stronger for women, who had received multiple smallpox vaccinations (odds ratio of 0.18 [95% CI, 0.05–0.64]).

We hypothesized that smallpox vaccination has a stronger protective effect against HIV-1 in women than men. If this is the case, the logical deduction is that the female/male HIV-1 prevalence ratio should increase for age groups with decreasing smallpox vaccination coverage while there would be no change in the female/male HIV-1 prevalence ratio for age groups with steady smallpox vaccination coverage. By using a female/male HIV-1 prevalence ratio, we could to some extent disregard time trends – such as the general spread of HIV-1 worldwide and the increased focus on prophylaxis and treatment – affecting both sexes and all age groups. We tested the hypothesis in two cohorts followed with sequential HIV surveys in Guinea-Bissau since the late 1980s.

Methods

In this ecological study, we compared the changes in smallpox vaccination coverage with the change in female/male HIV-1 prevalence ratio for the age groups that were between 15-34 (a decreasing smallpox vaccination coverage) and ≥ 35 years (a steady smallpox vaccination coverage) over a 30-year period. All data was based on aggregated data from different sources: We used

1
2 reported HIV-1 prevalence surveys in Bissau, the capital of Guinea-Bissau, and Caió, a rural district
3
4 of Guinea-Bissau.(4, 5) We used individually-based data from a smallpox vaccination scar survey
5
6 in Bissau in 2005 to model smallpox vaccination coverages at the time of the HIV-1 surveys.
7

8 9 **Patient and Public Involvement**

10
11 As this study was based on previously published data,(4-6) neither patients or the public were
12
13 involved in conducting this research.
14

15 16 **Estimates of smallpox vaccination coverage**

17
18 Smallpox vaccination typically leaves a distinct vaccination scar. We used a cohort of individuals,
19
20 who had both participated in a smallpox vaccination scar survey (2005-2007) and an HIV
21
22 prevalence survey (2004-2006) conducted in Bissau (previously published (6)) to model the
23
24 historical changes in smallpox vaccination coverage (see below). The smallpox vaccination scar
25
26 prevalence is comparable between urban and rural Guinea-Bissau.(7, 8) In the smallpox vaccination
27
28 scar survey, field workers examined vaccination scars and interviewed study participants. The field
29
30 workers examined upper arms for vaccination scars and registered up to a maximum of five scars.
31
32 Scars were classified as BCG, smallpox vaccination, or “uncertain”, based on size, color, and
33
34 general appearance of the scar.
35
36
37
38
39
40

41
42 For each individual in the smallpox scar survey, we calculated the age the individual would have
43
44 had in the different HIV survey years (1987, 1996, 2005 and 2016). We approximated the age-
45
46 standardized smallpox vaccination coverage overall and by sex in the years 1987, 1996, 2005 and
47
48 2016 in each age group (15-34 and ≥ 35 years) by dividing the number of individuals with a
49
50 smallpox vaccination scar by the total number of individuals in each group. The “15-34 years”
51
52 group was chosen as they have a declining smallpox vaccination coverage over the different HIV
53
54 surveys. The “ ≥ 35 years” group covered ages between 35 to 65 (oldest age registered) and had a
55
56 steady smallpox vaccination coverage over the different HIV surveys. The smallpox vaccination
57
58
59
60

1
2 coverage estimation for the age group ≥ 35 in 2016 was changed to ≥ 45 to ensure a steady smallpox
3
4 vaccination coverage.
5
6
7

8
9 A small validation study based on a city register of smallpox vaccination from Bissau showed a
10
11 sensitivity of 90% (95% CI, 80-95%) by using smallpox scars as proxies for registered smallpox
12
13 vaccinations (62 individuals had smallpox scars in community surveys out of 69 registered as
14
15 smallpox vaccinated in the city register).(7)
16
17

18 **Estimates of female/male HIV-1 prevalence ratios**

19
20 Three HIV-1 prevalence surveys were carried out in parallel both in Bissau (1987, 1996, 2006)(4)
21
22 and Caió (1990, 1997, 2007).(5) An additional survey was carried out in Bissau in 2016.(9) In these
23
24 surveys, all individuals aged 15 years or older from randomly selected households were interviewed
25
26 and tested for HIV provided consent. In Guinea-Bissau, injection drug use is virtually
27
28 nonexistent,(10) and blood transfusions have been screened for HIV since 1987 (4); thus, HIV-1 is
29
30 almost exclusively sexually transmitted.
31
32
33
34
35

36
37 The HIV-1 data were reported by sex and 10-year age groups from 15 years of age. Based on these
38
39 data, we constructed a dataset with the number of observed individuals by sex, age group [15-34;
40
41 ≥ 35] and HIV-1 status for each of the HIV surveys. The reason for the age cut-off of 35 years was
42
43 that the last smallpox vaccination campaign in Guinea-Bissau was in 1975 and pre-school children
44
45 were rarely vaccinated (7) resulting in a decreasing smallpox vaccination among 15-34-year-old
46
47 individuals across HIV survey years. The combined estimates for 2016 were based on Bissau, as no
48
49 HIV survey had been carried out in Caió; in this survey, the age range was changed to ≥ 45 to ensure
50
51 a steady smallpox vaccination coverage.
52
53
54
55
56

57
58 The female/male HIV-1 prevalence ratio in two specific age groups was of interest in itself, but
59
60 also, by using such as comparison, we could to some extent disregard time trends in the general

1
2 spread of HIV-1 worldwide and in the focus on prophylaxis and treatment, which would affect both
3
4 sexes and all age groups.
5
6
7
8

9 **Statistical analysis**

10
11 We used R 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria) to estimate the
12
13 female/male HIV-1 prevalence ratios among individuals 15-34 years and ≥ 35 years for each HIV
14
15 survey (confidence intervals were calculated using the “epitools” package for risk ratios). Individual
16
17 level data sets were reconstructed for the surveys based on the summary tables in (4, 5, 9). To
18
19 estimate the probability of data showing the observed trend in female/male HIV-1 prevalence by the
20
21 combined HIV survey years (1987-90, 1996-97, 2006-07) by chance, we fitted a logistic regression
22
23 on HIV-1 status depending on HIV survey year as a linear and quadratic effect, sex and the
24
25 interaction between a linear effect of HIV survey year and sex. The model was fitted separately for
26
27 the individuals aged 15-34 and ≥ 35 . We interpreted the p-value for the interaction between survey
28
29 year (assumed linear effect) and sex as a test for a homogeneous association between sex and a
30
31 linear change in HIV-1 prevalence across survey years.
32
33
34
35
36
37
38

39 **Results**

40
41
42 For the age group ≥ 35 years (≥ 45 years in 2016), the estimated smallpox vaccination coverage
43
44 was similar across all the HIV surveys (fluctuating between 66% and 77%, Figure 1). As expected,
45
46 the smallpox vaccination coverage decreased over HIV survey years for the age group 15-34 years
47
48 (from 62% in 1987 to 0% in 2016, Figure 1). There was no indication that the smallpox vaccination
49
50 coverage differed between women and men (Supplementary figures 1 and 2). The general
51
52 prevalence of HIV-1 among adults ≥ 15 years of age increased from 0% (0/649) in 1987 to 4.6%
53
54 (118/2548) in 2006 in Bissau and from 0.5% (14/2770) in 1990 to 3.6% (105/2895) in 2007 in Caió.
55
56
57
58 In 2016 in Bissau, the HIV-1 prevalence among adults over 15 was 4.0% (104/2601).
59
60

1
2
3
4 As seen in Table 1, there was an increase in the female/male HIV-1 prevalence ratio among
5
6 individuals 15-34 years from the earliest to the latest conducted HIV surveys, the pattern being
7
8 similar in Bissau and Caió. Combined, the female prevalence increased from 0.3% to 4.3% from
9
10 1987-1990 to 2006-07, whereas the male prevalence increased from 0.3% to 1.9% in the same
11
12 period. The female/male HIV-1 prevalence ratio was 1.00 (95% confidence interval (CI) 0.17-5.99)
13
14 in 1987-90, 1.16 (95% CI 0.69-1.93) in 1996-97 and 2.32 (95% CI 1.51-3.56) in 2006-07. In a
15
16 logistic regression, the interaction-test for a homogeneous association between sex and a linear
17
18 change in HIV-1 prevalence across survey years for the individuals aged 15-34 years gave a p-value
19
20 of 0.04. Latest in Bissau in 2016, the female/male HIV-1 prevalence ratio was further increased to
21
22 5.41 (95% CI 2.15-13.61).
23
24
25
26
27
28

29 The older age group with steady smallpox vaccination coverage had no increase in the female/male
30
31 HIV-1 prevalence ratio. Combined, the female prevalence increased from 0.7% to 5.0% from 1987-
32
33 1990 to 2006-07, whereas the male prevalence increased from 0.4% to 6.2% in the same period.
34
35 Thus, the female/male HIV-1 prevalence ratios were 1.93 (95% CI 0.40-9.25) in 1987-90, 1.32
36
37 (95% CI 0.83-2.10) in 1996-97, 0.81 (95% CI 0.56-1.16) in 2006-07. The test of interaction for a
38
39 homogeneous association between sex and a linear change in HIV-1 prevalence across surveys gave
40
41 a p-value of 0.07 and the direction trended towards the opposite direction than for the younger age
42
43 group. The female/male HIV-1 prevalence ratio was 1.03 (95% CI 0.47-2.25) in 2016 in Bissau.
44
45
46
47
48
49

50 The combined female/male HIV-1 prevalence ratios are illustrated in Figure 2. Relative to the F/M
51
52 prevalence ratio among the older age group, the F/M prevalence ratio in the 15-34 years age group
53
54 increased from 0.52 (95% CI 0.05-5.61) in 1987-90 to 0.88 (95% CI 0.44-1.75) in 1996-97 to 2.88
55
56 (95% CI 1.64-5.05) in 2006-07 to 5.26 (95% CI 1.57-17.65) in 2016 (2016 estimates were only
57
58 based on Bissau data) (ratios of ratios based on Table 1).
59
60

Discussion

As we had hypothesized the female/male HIV-1 prevalence ratio increased for the age group 15-34 years, as the proportion with smallpox vaccination scars decreased, whereas the female/male HIV-1 prevalence ratio remained unchanged for the age group ≥ 35 years, which had a steady smallpox vaccination coverage over the HIV-1 survey years.

Strengths and limitations

This study was based on information from large HIV surveys carried out over 20-30 years in two different settings, urban and rural Guinea-Bissau. As no central smallpox vaccination register exists in Guinea-Bissau, we used smallpox vaccination scars as a proxy for the smallpox vaccination coverage. We have previously shown that smallpox scars have a sensitivity of $>90\%$ in correctly identifying smallpox vaccinated individuals (no specificity measure available).⁽⁷⁾ Some BCG vaccination scars and accidental wounds may have been misclassified as smallpox vaccination scars, but misclassification is unlikely to be sex-differential. Potential variation in false-positive and false-negative rates of scar across surveys would likewise not be expected to be sex-differential. Participation in the HIV surveys varied only slightly across the survey years in Bissau, being 86% in 1987, 85% in 1996, 79% in 2006 and 83% in 2016; furthermore, the HIV prevalence in participants, who were easy to reach, was similar to the prevalence in those who were difficult to reach.^(4, 9) Hence, differential participation in different study years is unlikely to have caused selection bias.

The ecological design enabled us to investigate a potentially important hypothesis, but the results needs to be interpreted with caution since this design can be vulnerable to misinterpretations. By using the ratio of HIV-1 prevalence between sexes and within age groups, we could to some extent

1
2 disregard time trends such as the general spread of HIV-1 worldwide and the roll out of prophylaxis
3
4 and treatment affecting both sexes and all age groups.
5
6
7
8
9

10 11 **Female/male HIV-1 prevalence trends in Sub-Saharan Africa**

12
13 Consistent with our finding, cross sectional surveys from Malawi,(11) Zambia,(12) and South
14
15 Africa(13) show that the birth cohorts who are too young to have been smallpox vaccinated have an
16
17 increased female/male HIV prevalence compared with older birth cohorts, who are likely to have
18
19 been smallpox vaccinated before the worldwide phase-out in 1980.
20
21
22
23

24
25 Furthermore, UNAIDS data for the female/male HIV-1 prevalence from 1985 to 2003 in Sub-
26
27 Saharan Africa shows that in the 15-49-year-old age group, the number of HIV-1 affected women
28
29 began to increase over the number of men during the early 1990s.(14) The female/male HIV-1 ratio
30
31 increased at the same time as the smallpox vaccination coverage decreased after 1980. A multi-
32
33 country study using repeated national representative demographic and health surveys on HIV
34
35 prevalence in Sub-Saharan Africa during the 2000s did not find an increasing female/male HIV
36
37 prevalence ratio.(15) In contrast to the HIV surveys from Guinea-Bissau, where there was a clear
38
39 increase in the prevalence of HIV-1, the reported HIV prevalence generally decreased between
40
41 repeated surveys in other regions of Africa.(15) The female/male HIV prevalence ratio may be
42
43 influenced by multiple factors, and the introduction of HIV treatment, which only took place in the
44
45 late 2000s in Guinea-Bissau,(16) may have blurred the female/male HIV prevalence ratio trends in
46
47 the 2000s surveys from Sub-Saharan Africa.
48
49
50
51
52
53
54

55 **Potential causes of sex differences in HIV-1**

56
57 The sex differences in the susceptibility to HIV-1 could theoretically be due to physiological,
58
59 hormonal or local microbial differences, and higher prevalence of sexually transmitted diseases
60

1 causing a higher male-to-female than female-to-male HIV transmission rate.(14) These
2 explanations would however not explain why young women in Sub-Saharan Africa did not have a
3 higher HIV-1 prevalence than men when the HIV epidemic started. Our results showed that women
4 and men in the age group <35 years had similar HIV-1 prevalence in 1987-1990 of 0.3% but while
5 men's prevalence did not increase much in the younger age group, potentially due to more focus on
6 availability and use of condoms over time, women's prevalence continued to increase (Table 1). It
7 may be that female's increased susceptibility were neutralized by the smallpox vaccination and
8 became expressed when smallpox vaccination was stopped.

9
10
11
12
13
14
15
16
17
18
19
20
21
22 Alternative explanations for the sex-age-time pattern may be sought in social and cultural changes
23 over time, including gender-power imbalances.(14) Analyses of sexual mixing patterns from South
24 Africa(13) suggest that since young women often have sexual relations with older men, then as the
25 prevalence of HIV-1 increases among older men the prevalence among young women will follow.
26 We have no specific data to assess possible changes over time in the frequency of sexual relations
27 across age groups. All ethnic groups in Guinea-Bissau has a taboo on intercourse while the mother
28 is breastfeeding for 1½-3 years, which may have created a permissive attitude towards extra-marital
29 sexual relationships (possible causing sexual relations between older men and younger women). We
30 have documented such taboo on intercourse while breastfeeding and a permissive attitude back to
31 the 1980s in Guinea-Bissau (17) so it is clearly not a new phenomenon. While it is possible that
32 behavioral patterns became increasingly permissive of extra-marital sexual relationships in a setting
33 with rapidly increasing urbanization, it seems unlikely that the same change would have happened
34 in a rural setting. We find it unlikely that the similar pattern of increasing female/male HIV-1
35 prevalence ratio in both an urban and a rural setting can be explained merely by changes in sexual
36 behavior patterns. During the study period, there may have been increased awareness and
37 availability of condoms, but this would likely have affected the risk of acquiring HIV equally in
38 both sexes or if anything diminished the risk in females relative to males.

1
2
3
4 It should be noted, though, that the observation that the female/male HIV-1 prevalence ratio for the
5
6 age group 15-34 seems to continue to increase in Bissau with a ratio of 5.41 (95% CI 2.15-13.61) in
7
8 2016 compared with 2.34 (95% CI 1.35-4.04) in 2006, despite the prevalence of smallpox
9
10 vaccination coverage for this age group is likely to only have changed from 4% to 0%, suggests that
11
12 other factors continue to affect the susceptibility of HIV-1 differently for men and women.
13
14
15
16
17

18 **Biological mechanisms**

19
20 The CCR5 is fundamental for establishing HIV-1 infection.(18) The CCR5-delta-32 deletion
21
22 confers resistance to HIV-1 by preventing expression of the CCR5 receptor; this allele provides
23
24 almost complete resistance to HIV-1 in homozygous individuals.(18) A recent immunological study
25
26 found that cells from smallpox vaccinated individuals had up to 5-fold reduction in CCR-5 tropic
27
28 HIV-1 replication *in vitro*,(2) which supports a role for smallpox vaccine in HIV-1 prevention
29
30 through heterologous immunity. A recent study did not show an association between smallpox
31
32 vaccination scar and CCR5 expression on the surface of peripheral T-lymphocytes among HIV
33
34 seronegative women old enough to have had a chance of being smallpox vaccinated;(19) this may
35
36 be due to delay between smallpox vaccination and immunological testing of more than four decades
37
38 or that the smallpox-unvaccinated control group had received another immunomodulator, the BCG
39
40 vaccine.(20)
41
42
43
44
45
46
47

48 In animal models, administrating smallpox vaccination via skin scarification increased immune
49
50 response and survival compared with other modes of administration.(21) Murine studies have
51
52 shown that intradermal smallpox vaccination induced long-lived non-recirculating CD8+ skin
53
54 resident T-memory cells that resided within the entire skin and protected against reinfection.(22)
55
56 This indicates that vaccination can spread throughout the entire epithelial surface to create a
57
58 “shield” against infection.
59
60

1
2
3
4 Smallpox vaccine may also affect the innate immune system more broadly; in a very recent study,
5
6 human monocytes trained with smallpox vaccine showed significantly increased IL-6 and TNF- α
7
8 production to stimulation with non-related stimuli, compared to non-trained monocytes.(23)
9
10

11
12
13 Overall, there is some immunological evidence to support that smallpox vaccination can provide
14
15 cross-protection against HIV-1 infection. None of the above studies reported effects by sex, but it is
16
17 plausible that an epithelial protection might be particularly protective against vaginally acquired
18
19 HIV-1 infection.
20
21
22
23
24

25 **Conclusion**

26
27 Our hypothesis that termination of smallpox vaccination may have increased the female/male HIV-
28
29 1 prevalence ratio was compatible with our results. More research is needed to test this hypothesis,
30
31 and we hope other research groups will test the hypothesis and other potential explanations for the
32
33 change in female-male HIV prevalence ratios over time in individual-based data. While it may not
34
35 be possible to reintroduce smallpox vaccine, if more support for the hypothesis that smallpox
36
37 vaccine protected females against HIV can be obtained, from epidemiological and immunological
38
39 studies, it could provide important information for HIV-1 vaccine research.
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Author contact information:

Andreas Rieckmann, Bandim Health Project, OPEN, Institute of Clinical Research, University of Southern Denmark/Odense University Hospital, Odense, Denmark. Research Center for Vitamins and Vaccines (CVIVA), Bandim Health Project, Statens Serum Institut, Artillerivej 5, DK-2300 Copenhagen S, Denmark and Section of Epidemiology, Department of Public Health, University of Copenhagen, Copenhagen, Denmark. anri@ssi.dk

Marie Villumsen, Center for Clinical Research and Prevention, Bispebjerg and Frederiksberg Hospital, Capital Region, Copenhagen, Denmark. marie.villumsen@regionh.dk

Bo Langhoff Hønge, Department of Clinical Immunology, Aarhus University Hospital, Denmark and Bandim Health Project, Indepth Network, Apartado 861, Bissau, Guinea-Bissau. bohonge@gmail.com

Signe Sørup, Bandim Health Project, OPEN, Institute of Clinical Research, University of Southern Denmark/Odense University Hospital, Odense, Denmark. Research Center for Vitamins and Vaccines (CVIVA), Bandim Health Project, Statens Serum Institut, Artillerivej 5, DK-2300 Copenhagen S, Denmark and Department of Clinical Epidemiology, Aarhus University, Olof Palmes Allé 43-45, DK-8200 Aarhus N, Denmark. SGS@ssi.dk

Amabélia Rodrigues, Bandim Health Project, Indepth Network, Apartado 861, Bissau, Guinea-Bissau. a.rodrigues@bandim.org

Zacarias José da Silva, National Institute of Public Health (INASA), CP 1013, Bissau, Guinea-Bissau. zacarias55@hotmail.com

Hilton Whittle, London School of Hygiene and Tropical Medicine, Keppel Street, London, UK, hcwhittle@yahoo.co.uk

Christine Stabell Benn, Bandim Health Project, OPEN, Institute of Clinical Research, University of Southern Denmark/Odense University Hospital, Odense, Denmark. Research Center for Vitamins and Vaccines (CVIVA), Bandim Health Project, Statens Serum Institut, Artillerivej 5, DK-2300

1
2 Copenhagen S, Denmark and OPEN, Odense University Hospital/Institute of Clinical Research,
3
4 University of Southern Denmark, Odense, Denmark. CB@ssi.dk

5
6 Peter Aaby, Bandim Health Project, Indepth Network, Apartado 861, Bissau, Guinea-Bissau.

7
8
9 p.aaby@bandim.org

10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

References

1. Mayr A. Taking advantage of the positive side-effects of smallpox vaccination. *Journal of veterinary medicine B, Infectious diseases and veterinary public health*. 2004;51(5):199-201.
2. Weinstein RS, Weinstein MM, Alibek K, Bukrinsky MI, Brichacek B. Significantly reduced CCR5-tropic HIV-1 replication in vitro in cells from subjects previously immunized with Vaccinia Virus. *BMC immunology*. 2010;11:23.
3. Rieckmann A, Villumsen M, Jensen ML, Ravn H, Silva ZJd, Sørup S, et al. The effect of smallpox and BCG vaccination on the risk of HIV-1 infection in Guinea-Bissau and Denmark. *Open Forum Infect Dis*. 2017.
4. da Silva ZJ, Oliveira I, Andersen A, Dias F, Rodrigues A, Holmgren B, et al. Changes in prevalence and incidence of HIV-1, HIV-2 and dual infections in urban areas of Bissau, Guinea-Bissau: is HIV-2 disappearing? *Aids*. 2008;22(10):1195-202.
5. Tienen C, van der Loeff MS, Zaman SM, Vincent T, Sarge-Njie R, Peterson I, et al. Two distinct epidemics: the rise of HIV-1 and decline of HIV-2 infection between 1990 and 2007 in rural Guinea-Bissau. *Journal of acquired immune deficiency syndromes*. 2010;53(5):640-7.
6. Rieckmann A, Villumsen M, Jensen ML, Ravn H, da Silva ZJ, Sorup S, et al. The Effect of Smallpox and Bacillus Calmette-Guerin Vaccination on the Risk of Human Immunodeficiency Virus-1 Infection in Guinea-Bissau and Denmark. *Open forum infectious diseases*. 2017;4(3):ofx130.
7. Aaby P, Gustafson P, Roth A, Rodrigues A, Fernandes M, Sodemann M, et al. Vaccinia scars associated with better survival for adults. An observational study from Guinea-Bissau. *Vaccine*. 2006;24(29-30):5718-25.
8. Jensen ML, Dave S, Schim van der Loeff M, da Costa C, Vincent T, Leligdowicz A, et al. Vaccinia scars associated with improved survival among adults in rural Guinea-Bissau. *PloS one*. 2006;1:e101.
9. Olesen JS, Jespersen S, da Silva ZJ, Rodrigues A, Erikstrup C, Aaby P, et al. HIV-2 continues to decrease, whereas HIV-1 is stabilizing in Guinea-Bissau. *Aids*. 2018;32(9):1193-8.
10. Månsson F. HIV-1, HIV-2, and other Sexually Transmitted Infections in GuineaBissau, West Africa. Lund University. 2012.
11. Maman D, Chilima B, Masiku C, Ayouba A, Masson S, Szumilin E, et al. Closer to 90-90-90. The cascade of care after 10 years of ART scale-up in rural Malawi: a population study. *Journal of the International AIDS Society*. 2016;19(1):20673.
12. Chanda-Kapata P, Kapata N, Klinkenberg E, William N, Mazyanga L, Musukwa K, et al. The adult prevalence of HIV in Zambia: results from a population based mobile testing survey conducted in 2013-2014. *AIDS research and therapy*. 2016;13:4.
13. de Oliveira T, Kharsany AB, Graf T, Cawood C, Khanyile D, Grobler A, et al. Transmission networks and risk of HIV infection in KwaZulu-Natal, South Africa: a community-wide phylogenetic study. *The lancet HIV*. 2017;4(1):e41-e50.
14. Quinn TC, Overbaugh J. HIV/AIDS in women: an expanding epidemic. *Science*. 2005;308(5728):1582-3.
15. Hegdahl HK, Fylkesnes KM, Sandoy IF. Sex differences in HIV prevalence persist over time: Evidence from 18 countries in Sub-Saharan Africa. *PloS one*. 2016;11(2):e0148502.
16. Jespersen S, Honge BL, Oliveira I, Medina C, da Silva Te D, Correia FG, et al. Challenges facing HIV treatment in Guinea-Bissau: the benefits of international research collaborations. *Bulletin of the World Health Organization*. 2014;92(12):909-14.
17. Høgsborg M, Aaby P. Sexual relations, use of condoms and perceptions of AIDS in an urban area of Guinea-Bissau with a high prevalence of HIV-2. *Sexual behaviour and networking: anthropological and socio-cultural studies on the transmission of HIV*, edited by Tim Dyson Liege, Belgium, Editions Derouaux-Ordina, [1992] 203-31. 1992.
18. Dean M, Carrington M, Winkler C, Huttley GA, Smith MW, Allikmets R, et al. Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. *Hemophilia Growth and Development Study, Multicenter AIDS Cohort Study, Multicenter Hemophilia Cohort Study, San Francisco City Cohort, ALIVE Study*. *Science*. 1996;273(5283):1856-62.
19. Beck KB, Honge BL, Olesen JS, Petersen MS, Jespersen S, Wejse C, et al. Long-term effects of smallpox vaccination on expression of the HIV-1 co-receptor CCR5 in women. *PloS one*. 2018;13(11):e0207259.
20. Netea MG, Joosten LA, Latz E, Mills KH, Natoli G, Stunnenberg HG, et al. Trained immunity: A program of innate immune memory in health and disease. *Science*. 2016;352(6284):aaf1098.
21. Rice AD, Adams MM, Lindsey SF, Swetnam DM, Manning BR, Smith AJ, et al. Protective properties of vaccinia virus-based vaccines: skin scarification promotes a nonspecific immune response that protects against orthopoxvirus disease. *Journal of virology*. 2014;88(14):7753-63.
22. Jiang X, Clark RA, Liu L, Wagers AJ, Fuhlbrigge RC, Kupper TS. Skin infection generates non-migratory memory CD8+ T(RM) cells providing global skin immunity. *Nature*. 2012;483(7388):227-31.

1
2 23. Blok BA, Jensen KJ, Aaby P, Fomsgaard A, van Crevel R, Benn CS, et al. Opposite effects of Vaccinia
3 and modified Vaccinia Ankara on trained immunity. *European journal of clinical microbiology & infectious diseases* :
4 official publication of the European Society of Clinical Microbiology. 2019;38(3):449-56.
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

1
2 **Figure 1. Estimated smallpox vaccination scar prevalence in different age groups for each**
3
4 **sequential HIV survey**
5

6
7
8 Insert figure 1
9

10 Based on data from Bissau, Guinea-Bissau, previously published.(6) The estimation for the age
11 group ≥ 35 in 2016 was changed to ≥ 45 to ensure a steady smallpox vaccination coverage.
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46

1 **Table 1. The female/male HIV-1 prevalence ratio (PR) by age group, survey year and study site**

Age group	15-34 years (decreasing smallpox vaccination rates in later years; see Figure 1)				≥35 years (steady smallpox vaccination rate over time; see Figure 1)			
	Estimated smallpox coverage	HIV-1 prevalence (%)		Female/male PR (95% CI)	Estimated smallpox coverage	HIV-1 prevalence (%)		Female/male PR (95% CI)
Study site and survey year		Female	Male			Female	Male	
Caió								
1990	62%	0.3% (3/861)	0.4% (2/541)	0.94 (0.16-5.62)	72%	0.8% (7/907)	0.4% (2/461)	1.78 (0.37-5.53)
1997	27%	1.8% (17/958)	1.9% (14/738)	0.94 (0.46-1.89)	77%	4.4% (41/943)	2.8% (13/471)	1.58 (0.85-2.91)
2007	4%	3.2% (28/885)	1.5% (11/742)	2.13 (1.07-4.26)	71%	4.8% (41/850)	6.0% (25/418)	0.81 (0.50-1.31)
Bissau								
1987	62%	0% (0/243)	0% (0/197)	NA	72%	0% (0/110)	0% (0/99)	NA
1996	27%	2.2% (19/881)	1.5% (10/680)	1.47 (0.69-3.13)	77%	3.3% (13/394)	3.5% (12/346)	0.95 (0.44-2.06)
2006	4%	5.3% (56/1056)	2.3% (16/705)	2.34 (1.35-4.04)	71%	5.4% (25/466)	6.5% (21/321)	0.82 (0.47-1.44)
2016	0%	4.2% (41/983)	0.8% (5/648)	5.41 (2.15-13.61)	66%	5.2% (13/252)**	5.0% (11/219)**	1.03 (0.42-2.25)**
Combined								
1987-90	62%	0.3% (3/1104)	0.3% (2/738)	1.00 (0.17-5.99)	72%	0.7% (7/1017)	0.4% (2/560)	1.93 (0.40-2.25)
1996-97	27%	2.0% (36/1839)	1.7% (24/1418)	1.16 (0.69-1.93)	77%	4.0% (54/1337)	3.1% (25/817)	1.32 (0.83-2.10)

2006-07	4%	4.3% (84/1941)	1.9% (27/1447)	2.32 (1.51-3.56)	71%	5.0% (66/1316)	6.2% (46/739)	0.81 (0.56-1.16)
---------	----	-------------------	-------------------	------------------	-----	-------------------	------------------	------------------

2 Data are extracted from (4, 5, 9).

3 * This estimate is only based on information from Bissau.

4 ** The estimation for the age group ≥ 35 in 2016 was changed to ≥ 45 to ensure a steady smallpox vaccination coverage.

For peer review only

1
2
3 **Figure 2. Female/male HIV-1 prevalence ratios, Bissau and Caió**
4

5
6 Insert figure 2
7

8
9 Visualisations of estimates from Table 1. Circles and triangles represent point estimates and lines
10 represent the 95% confidence intervals. The estimation for the age group ≥ 35 in 2016 was only
11 from Bissau and was changed to ≥ 45 to ensure a steady smallpox vaccination coverage.
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

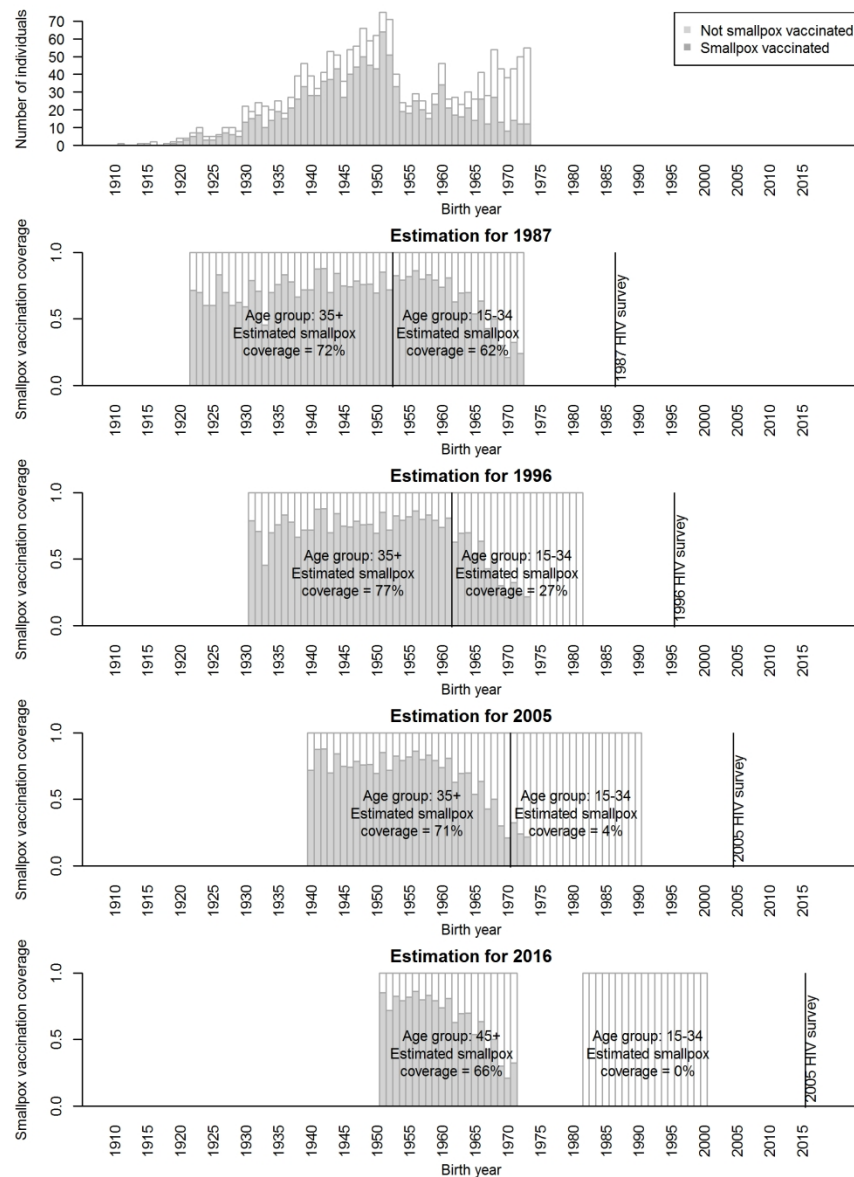


Figure 1. Estimated smallpox vaccination scar prevalence in different age groups for each sequential HIV survey.

Based on data from Bissau, Guinea-Bissau, previously published.⁽⁶⁾ The estimation for the age group ≥ 35 in 2016 was changed to ≥ 45 to ensure a steady smallpox vaccination coverage

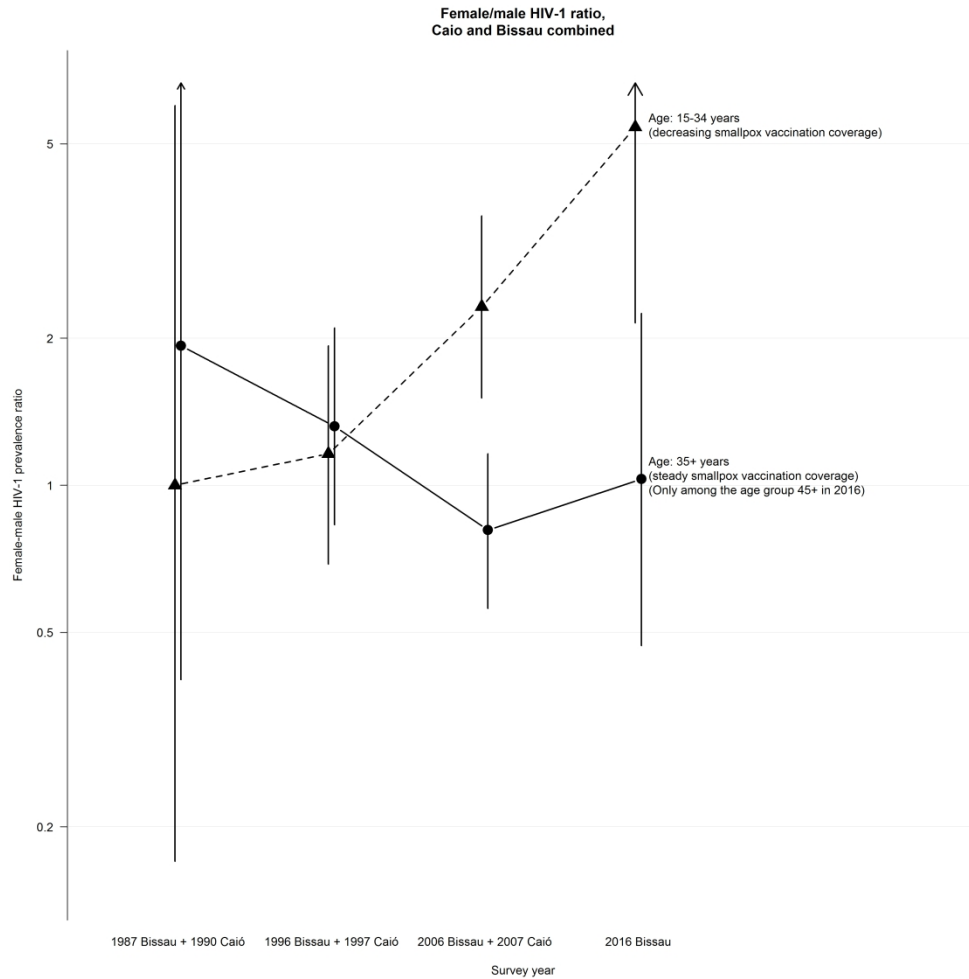
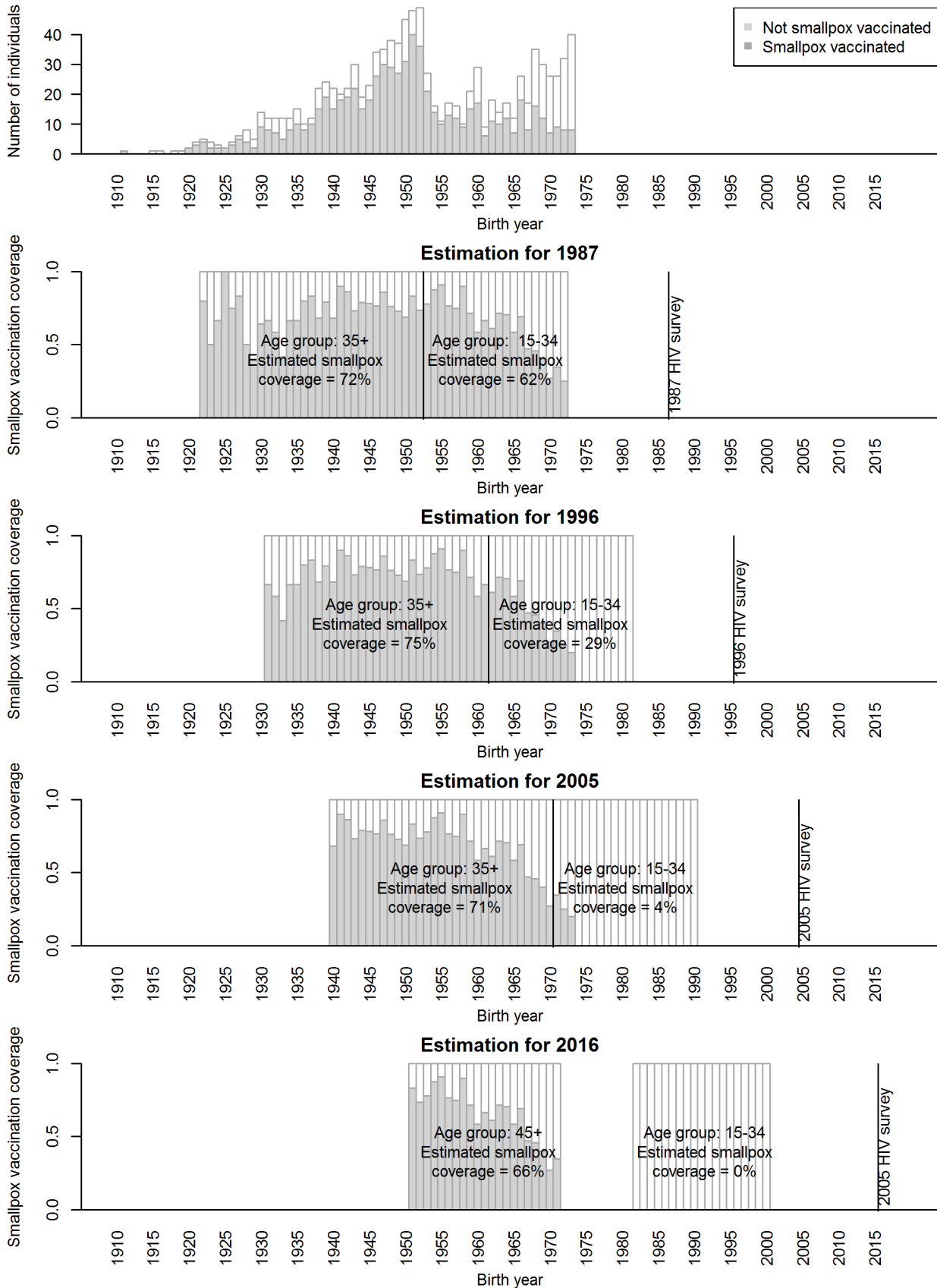


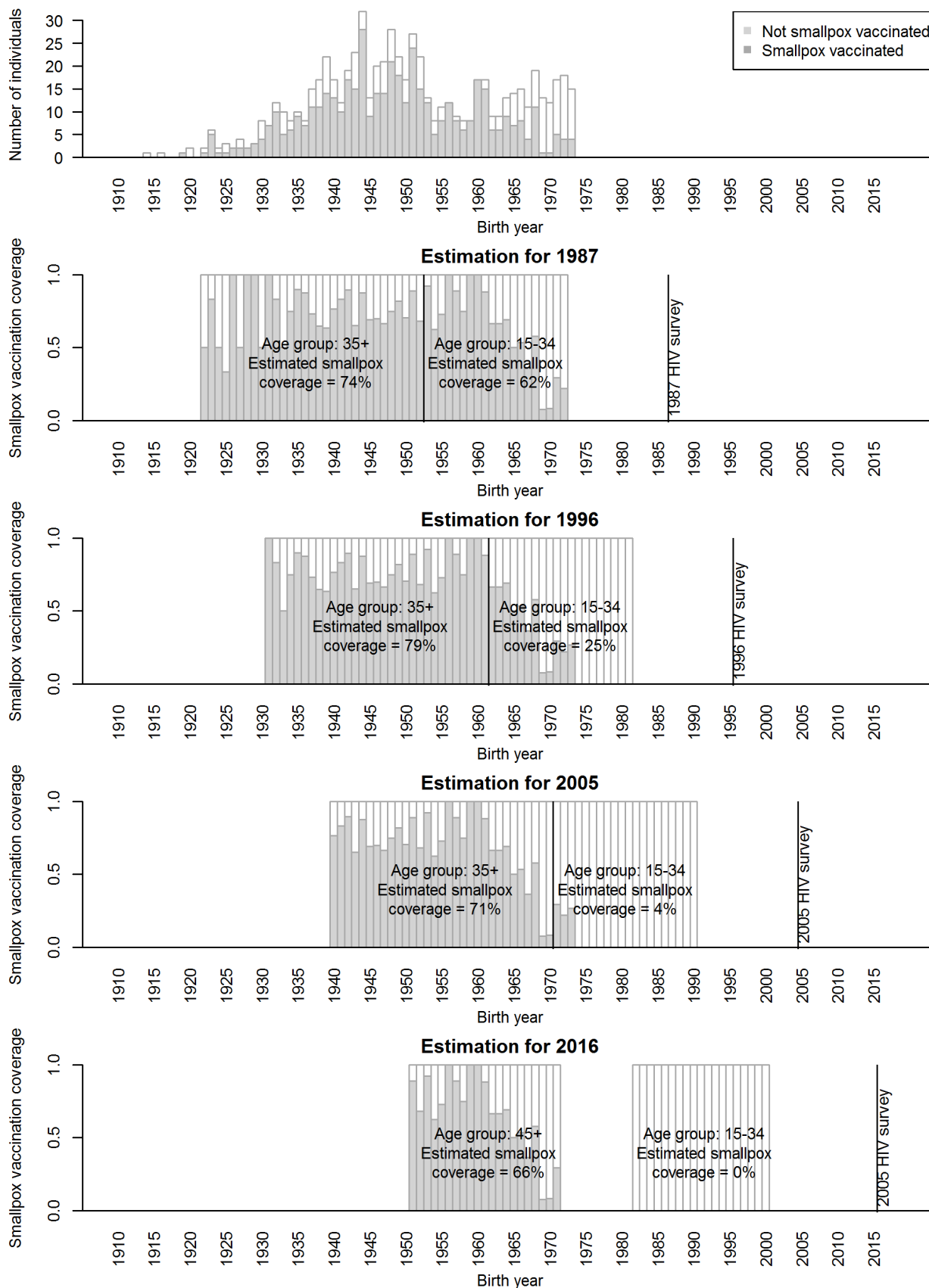
Figure 2. Female/male HIV-1 prevalence ratios, Bissau and Caió.

Visualisations of estimates from Table 1. Circles and triangles represent point estimates and lines represent the 95% confidence intervals. The estimation for the age group ≥ 35 in 2016 was only from Bissau and was changed to ≥ 45 to ensure a steady smallpox vaccination coverage.

Supplementary figure 1. Estimated smallpox vaccination scar prevalence in different age groups for each sequential HIV survey among women



Supplementary figure 2. Estimated smallpox vaccination scar prevalence in different age groups for each sequential HIV survey among men



1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60STROBE Statement—Checklist of items that should be included in reports of *cross-sectional studies*

	Item No	Recommendation	Responses
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	✓
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	✓
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	✓
Objectives	3	State specific objectives, including any prespecified hypotheses	✓
Methods			
Study design	4	Present key elements of study design early in the paper	✓
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	✓
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants	✓
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	✓
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	✓
Bias	9	Describe any efforts to address potential sources of bias	✓ (these are discussed)
Study size	10	Explain how the study size was arrived at	✓
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	✓
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	✓
		(b) Describe any methods used to examine subgroups and interactions	✓
		(c) Explain how missing data were addressed	✓
		(d) If applicable, describe analytical methods taking account of sampling strategy	NA, as the study builds upon published data
		(e) Describe any sensitivity analyses	✓
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	✓ (this is an ecological study)
		(b) Give reasons for non-participation at each stage	✓ (this is an ecological study)

		(c) Consider use of a flow diagram	✓ (this is an ecological study, and we chose not to include a flow chart)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	✓
		(b) Indicate number of participants with missing data for each variable of interest	✓
Outcome data	15*	Report numbers of outcome events or summary measures	✓
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	✓ (the comparison group above 35 years function an adjustment of calendar time)
		(b) Report category boundaries when continuous variables were categorized	NA
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	✓ (we report relative risks and absolute values)
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	✓ (all analyses are presented)
Discussion			
Key results	18	Summarise key results with reference to study objectives	✓
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	✓
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	✓
Generalisability	21	Discuss the generalisability (external validity) of the study results	✓
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	✓

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

The phase-out of smallpox vaccination and the female/male HIV-1 prevalence ratio: an ecological study from Guinea-Bissau

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2019-031415.R2
Article Type:	Original research
Date Submitted by the Author:	06-Sep-2019
Complete List of Authors:	Rieckmann, Andreas; Bandim Health Project, Statens Serum Institut Villumsen, Marie Hønge, Bo; Aarhus University Hospital, Department of Infectious Diseases Sørup, Signe Rodrigues, Amabelia; Bandim Health Project, Bandim Health Project, da Silva, Zacarias Whittle, Hilton; London School of Hygiene and Tropical Medicine, Benn, Christine; Statens Serum Institut, Bandim Health Project; University of Southern Denmark, OPEN Aaby, Peter; Bandim Health Project,
Primary Subject Heading:	HIV/AIDS
Secondary Subject Heading:	Public health
Keywords:	Heterologous immunity, HIV-1, Non-specific effects of vaccines, Smallpox vaccination, Vaccinia

SCHOLARONE™
Manuscripts

The phase-out of smallpox vaccination and the female/male HIV-1 prevalence ratio: an ecological study from Guinea-Bissau

Andreas Rieckmann^{1,2,3}*, Marie Villumsen⁴, Bo Langhoff Hønge^{5,6}, Signe Sørup^{1,2,7}, Amabélia Rodrigues⁶, Zacarias José da Silva⁸, Hilton Whittle⁹, Christine Stabell Benn^{1,2}, Peter Aaby⁶

¹ Bandim Health Project, OPEN, Institute of Clinical Research, University of Southern Denmark/Odense University Hospital, Odense, Denmark.

² Research Center for Vitamins and Vaccines (CVIVA), Bandim Health Project, Statens Serum Institut, Artillerivej 5, DK-2300 Copenhagen S, Denmark

³ Section of Epidemiology, Department of Public Health, University of Copenhagen, Copenhagen, Denmark

⁴ Center for Clinical Research and Prevention, Bispebjerg and Frederiksberg Hospital, Capital Region, Copenhagen, Denmark

⁵ Department of Clinical Immunology, Aarhus University Hospital, Denmark

⁶ Bandim Health Project, Indepth Network, Apartado 861, Bissau, Guinea-Bissau

⁷ Department of Clinical Epidemiology, Aarhus University, Olof Palmes Allé 43-45, DK-8200 Aarhus N, Denmark

⁸ National Institute of Public Health (INASA), CP 1013, Bissau, Guinea-Bissau

⁹ London School of Hygiene and Tropical Medicine, Keppel Street, London, UK

* Corresponding author: Andreas Rieckmann, anri@ssi.dk

Word count: Abstract: 298, Manuscript 2942, References 23

Tables: 1, Figures: 2, Supplementary figures 2.

1
2 **Financial support:** The Danish National Research Foundation (DNRF) supported the establishment
3
4 of Research Center for Vitamins and Vaccines [DNRF108]. AR was supported by an unrestricted
5
6 Faculty of Health Sciences-scholarship from University of Southern Denmark. SS was supported by
7
8 a grant from the Danish Council for Independent Research [DFR – 4183-00316].
9

10
11 **Disclosures:** The authors have no conflicts of interest.
12

13 **Ethics statement:** Our study is based on published results from 3 original research papers. The
14
15 study by da Silva et al. was approved by the Guinea-Bissau Government Ethics Committee and the
16
17 Danish Central Scientific Ethics Committee. The study by van Tienen was approved by the Gambia
18
19 Government/MRC Laboratories Joint Ethics Committee and by the Ministry of Health of Guinea-
20
21 Bissau. The study by Olesen et al. was approved by the National Research Ethics Committee in
22
23 Guinea-Bissau and received consultative approval from the National Research Ethics Committee of
24
25 Denmark.
26
27

28
29 **Data availability statement:** Information about HIV-1 was extracted from published results from
30
31 original research papers carried out in parallel both in Bissau (1987, 1996, 2006 [da Silva et al],
32
33 2016 [Olesen et al.]) and Caió (1990, 1997, 2007 [van Tienen et al.]) in Guinea-Bissau. Information
34
35 about smallpox vaccination was based on data from a cohort of individuals, who had both
36
37 participated in a smallpox vaccination scar survey (2005-2007) and an HIV prevalence survey
38
39 (2004-2006) conducted in Bissau. The Bandim Health Project (bandim@ssi.dk) can be contacted
40
41 for data requests.
42
43
44
45
46
47

48 **Author's contributions :** AnR and PA designed the study and drafted the manuscript. AnR, MV,
49
50 BLH, SS, AmR, ZJS, HW, CSB, and PA have substantially contributed to the analysis and
51
52 interpretation of the results. BLH and ZJS designed and acquired data with regards to the original
53
54 papers of which data is reanalyzed in this paper.. All authors have revised the paper critically and
55
56 approved the final version.
57
58
59
60

Abstract

Objective: In Guinea-Bissau, West Africa, we observed that having a smallpox vaccination scar was associated with lower HIV-1 prevalence, more strongly for women than men. If this represents a causal effect, the female/male HIV-1 prevalence ratio would increase for birth cohorts no longer receiving smallpox vaccination due to the phase-out of this vaccine.

Design: An ecological design using HIV surveys and information about smallpox vaccination coverage.

Setting: Urban and rural Guinea-Bissau.

Participants: Participants in HIV surveys were grouped into an age group with decreasing smallpox vaccination coverage (15-34 years) and an age group with steady smallpox vaccination coverage (≥ 35 years).

Interventions: The exposure of interest was the phase-out of the smallpox vaccine in Guinea-Bissau.

Primary and secondary outcome measures: HIV-1 prevalence.

Results: At both sites, the female/male HIV-1 prevalence ratio increased by calendar time for the age group with decreasing smallpox vaccination coverage; the combined female/male HIV-1 prevalence ratio among 15-34-year-olds was 1.00 (95% confidence interval (CI) 0.17-5.99) in 1987-90, 1.16 (95% CI 0.69-1.93) in 1996-97, 2.32 (95% CI 1.51-3.56) in 2006-07 (p-value for no trend=0.04). There was no increase in the female-male HIV-1 prevalence ratio for the age group ≥ 35 years with steady smallpox vaccination coverage; 1.93 (95% CI 0.40-9.25) in 1987-90, 1.32 (95% CI 0.83-2.10) in 1996-97, 0.81 (95% CI 0.56-1.16) in 2006-07 (p-value for no trend=0.07).

Conclusions: Thus, data was compatible with the deduction that the phase-out of smallpox vaccination may have increased the susceptibility to HIV-1 relatively more for women than men. Hence, phasing out smallpox vaccination may have contributed to the global increase in the female/male HIV-1 prevalence ratio among young individuals. Due to the potential fallacies of

1
2 ecological studies, the results should be interpreted carefully, and this hypothesis needs further
3
4 assessment. If the hypothesis is true, studies of smallpox vaccination could inform HIV-1 vaccine
5
6 research.
7
8
9

10
11 Key words: Heterologous immunity; HIV-1; Non-specific effects of vaccines; Smallpox
12
13 vaccination; Vaccinia.
14
15
16
17
18
19

20 21 Article summary

22 23 Strengths and limitations of this study

- 24
25
26 • The ecological design allowed us to assess a deduction of the hypothesis: Smallpox vaccination has a
27 protective effect against HIV-1, which is stronger among women than among men.
- 28
29 • To increase the robustness of the results, we utilized parallel data from both urban and rural Guinea-Bissau.
- 30
31 • Ecological studies should be interpreted carefully as spurious associations can arise, and thus triangulation
32
33 with existing studies are necessary to further assess this hypothesis.
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Introduction

Vaccination against smallpox infections was stopped globally in 1980 following the eradication of smallpox in 1977. It has been reported that smallpox vaccination reduced susceptibility to unrelated infectious diseases,(1) and in immunological *in vitro* studies, smallpox vaccination was associated with an up to 5-fold reduction in C-C chemokine receptor 5 (CCR5) tropic HIV-1 replication.(2) Based on vaccination scar readings in Guinea-Bissau and school health records in Denmark, we have shown that smallpox vaccination (and Bacille Calmette-Guérin vaccination [BCG]) was associated with a lower risk of HIV-1.(3) The adjusted odds ratio for HIV-1 infection was 0.52 (95% CI 0.32-0.84) for women and 0.77 (95% CI 0.48-1.24) for men. This association was stronger for women, who had received multiple smallpox vaccinations (odds ratio of 0.18 [95% CI, 0.05–0.64]).

We hypothesized that smallpox vaccination has a stronger protective effect against HIV-1 in women than men. If this is the case, a logical deduction is that the female/male HIV-1 prevalence ratio should increase for age groups with decreasing smallpox vaccination coverage while there would be no change in the female/male HIV-1 prevalence ratio for age groups with steady smallpox vaccination coverage. By using a female/male HIV-1 prevalence ratio, we could to some extent disregard calendar time trends – such as the general spread of HIV-1 worldwide and the increased focus on prophylaxis and treatment – affecting both sexes and all age groups. We tested the hypothesis in two cohorts followed with sequential HIV surveys in Guinea-Bissau since the late 1980s.

Methods

In this ecological study, we compared the changes in smallpox vaccination coverage with the change in female/male HIV-1 prevalence ratio for the age groups that were between 15-34 (a decreasing smallpox vaccination coverage) and ≥ 35 years (a steady smallpox vaccination coverage)

1
2 over a 30-year period. All analyses were based on aggregated data from different sources: We used
3
4 reported HIV-1 prevalence surveys in Bissau, the capital of Guinea-Bissau, and Caió, a rural district
5
6 of Guinea-Bissau.(4, 5) We used individually-based data from a smallpox vaccination scar survey
7
8 in Bissau in 2005 to model smallpox vaccination coverages at the time of the HIV-1 surveys.
9

10 **Patient and Public Involvement**

11
12 As this study was based on previously published data,(4-6) neither patients or the public were
13
14 involved in conducting this research.
15
16

17 **Estimates of smallpox vaccination coverage**

18
19 Smallpox vaccination typically leaves a distinct vaccination scar. We used a cohort of individuals,
20
21 who had both participated in a smallpox vaccination scar survey (2005-2007) and an HIV
22
23 prevalence survey (2004-2006) conducted in Bissau (previously published (6)) to model the
24
25 historical changes in smallpox vaccination coverage (see below). The smallpox vaccination scar
26
27 prevalence is comparable between urban and rural Guinea-Bissau.(7, 8) In the smallpox vaccination
28
29 scar survey, field workers examined vaccination scars and interviewed study participants. The field
30
31 workers examined the study participants' upper arms for vaccination scars and registered up to five
32
33 scars. Scars were classified as BCG, smallpox vaccination, or "uncertain", based on size, colour,
34
35 and general appearance of the scar.
36
37
38
39
40
41
42

43 For each individual in the smallpox scar survey, we calculated the age the individual would have
44
45 had in the different HIV survey years (1987, 1996, 2005 and 2016). We approximated the age-
46
47 standardized smallpox vaccination coverage overall and by sex for the years 1987, 1996, 2005 and
48
49 2016 in each age group (15-34 and ≥ 35 years) by dividing the number of individuals with a
50
51 smallpox vaccination scar by the total number of individuals in each group. The "15-34 years"
52
53 group was chosen as they have a declining smallpox vaccination coverage over the different HIV
54
55 surveys. The " ≥ 35 years" group covered ages between 35 to 65 (oldest age registered) and had a
56
57 steady smallpox vaccination coverage over the different HIV surveys. The smallpox vaccination
58
59
60

1
2 coverage estimation for the age group ≥ 35 in 2016 was changed to ≥ 45 to ensure a steady smallpox
3
4 vaccination coverage.
5
6
7

8
9 A small validation study based on a city register of smallpox vaccination from Bissau showed a
10
11 sensitivity of 90% (95% CI, 80-95%) by using smallpox scars as proxies for registered smallpox
12
13 vaccinations (62 individuals had smallpox scars in community surveys out of 69 registered as
14
15 smallpox vaccinated in the city register).(7)
16
17

21 **Estimates of female/male HIV-1 prevalence ratios**

22
23 Three HIV-1 prevalence surveys were carried out in parallel both in Bissau (1987, 1996, 2006)(4)
24
25 and Caió (1990, 1997, 2007).(5) An additional survey was carried out in Bissau in 2016.(9) In these
26
27 surveys of randomly selected households, all individuals aged 15 years or older were interviewed
28
29 and tested for HIV provided they accepted the informed consent. In Guinea-Bissau, injection drug
30
31 use is virtually non-existent,(10) and blood transfusions have been screened for HIV since 1987 (4);
32
33 thus, HIV-1 is almost exclusively sexually transmitted.
34
35
36
37
38

39 The results of the HIV-1 surveys were reported by sex and by 10-year age groups from 15 years of
40
41 age. Based on these data, we constructed a dataset with the number of observed individuals by sex,
42
43 age group [15-34; ≥ 35] and HIV-1 status for each of the HIV surveys. The reason for the age cut-
44
45 off of 35 years was that the last smallpox vaccination campaign in Guinea-Bissau was in 1975 and
46
47 pre-school children were rarely vaccinated (7) resulting in a decreasing smallpox vaccination
48
49 among 15-34-year-old individuals across HIV survey years. The combined estimates for 2016 were
50
51 only based on Bissau, as no HIV survey had been carried out in Caió; in this survey, the age range
52
53 was changed to ≥ 45 to ensure a steady smallpox vaccination coverage.
54
55
56
57
58
59
60

1
2 The female/male HIV-1 prevalence ratio in two specific age groups was of interest in itself, but
3
4 also, by using such as comparison, we could to some extent disregard calendar time trends such as
5
6 the spread, prophylaxis and treatment of HIV-1, which would affect both sexes and all age groups.
7
8
9

10 11 **Statistical analysis**

12
13 We used R 3.3.1 to estimate the female/male HIV-1 prevalence ratios among individuals 15-34
14
15 years and ≥ 35 years for each HIV survey (confidence intervals were calculated using the “epitools”
16
17 R package for risk ratios). Individual level-data sets were reconstructed for the surveys based on the
18
19 summary tables in (4, 5, 9). To estimate the probability of data showing the observed trend in
20
21 female/male HIV-1 prevalence by the combined HIV survey years (1987-90, 1996-97, 2006-07) by
22
23 chance, we fitted a logistic regression on HIV-1 status depending on HIV survey year as a linear
24
25 and quadratic effect, sex and the interaction between a linear effect of HIV survey year and sex. The
26
27 model was fitted separately for the individuals aged 15-34 and ≥ 35 . We interpreted the p-value for
28
29 the interaction between survey year (assumed linear effect) and sex as a test for a homogeneous
30
31 association between sex and a linear change in HIV-1 prevalence across survey years.
32
33
34
35
36
37
38

39 **Results**

40
41
42 For the age group ≥ 35 years (≥ 45 years in 2016), the estimated smallpox vaccination coverage
43
44 was similar across all the HIV surveys (fluctuating between 66% and 77%, Figure 1). As expected,
45
46 the smallpox vaccination coverage decreased over HIV survey years for the age group 15-34 years
47
48 (from 62% in 1987 to 0% in 2016, Figure 1). There was no indication that the smallpox vaccination
49
50 coverage differed between women and men (Supplementary figures 1 and 2). The general
51
52 prevalence of HIV-1 among adults ≥ 15 years of age increased from 0% (0/649) in 1987 to 4.6%
53
54 (118/2548) in 2006 in Bissau and from 0.5% (14/2770) in 1990 to 3.6% (105/2895) in 2007 in Caió.
55
56
57 In 2016 in Bissau, the HIV-1 prevalence among adults over 15 was 4.0% (104/2601).
58
59
60

1
2
3
4 As seen in Table 1, there was an increase in the female/male HIV-1 prevalence ratio among
5
6 individuals 15-34 years from the earliest to the latest conducted HIV surveys, the pattern being
7
8 similar in Bissau and Caió. Combined, the female prevalence increased from 0.3% to 4.3% from
9
10 1987-1990 to 2006-07, whereas the male prevalence increased from 0.3% to 1.9% in the same
11
12 period. The female/male HIV-1 prevalence ratio was 1.00 (95% confidence interval (CI) 0.17-5.99)
13
14 in 1987-90, 1.16 (95% CI 0.69-1.93) in 1996-97 and 2.32 (95% CI 1.51-3.56) in 2006-07. In a
15
16 logistic regression, the interaction-test for a homogeneous association between sex and a linear
17
18 change in HIV-1 prevalence across survey years for the individuals aged 15-34 years gave a p-value
19
20 of 0.04. Latest in Bissau in 2016, the female/male HIV-1 prevalence ratio was further increased to
21
22 5.41 (95% CI 2.15-13.61).
23
24
25
26
27
28

29
30 The older age group with steady smallpox vaccination coverage had no increase in the female/male
31
32 HIV-1 prevalence ratio. Combined, the female prevalence increased from 0.7% to 5.0% from 1987-
33
34 1990 to 2006-07, whereas the male prevalence increased from 0.4% to 6.2% in the same period.
35
36 Thus, the female/male HIV-1 prevalence ratios were 1.93 (95% CI 0.40-9.25) in 1987-90, 1.32
37
38 (95% CI 0.83-2.10) in 1996-97, 0.81 (95% CI 0.56-1.16) in 2006-07. The test of interaction for a
39
40 homogeneous association between sex and a linear change in HIV-1 prevalence across surveys gave
41
42 a p-value of 0.07 and the direction trended towards the opposite direction than for the younger age
43
44 group. The female/male HIV-1 prevalence ratio was 1.03 (95% CI 0.47-2.25) in 2016 in Bissau.
45
46
47
48
49

50
51 The combined female/male HIV-1 prevalence ratios are illustrated in Figure 2. Relative to the F/M
52
53 prevalence ratio among the older age group, the F/M prevalence ratio in the 15-34 years age group
54
55 increased from 0.52 (95% CI 0.05-5.61) in 1987-90 to 0.88 (95% CI 0.44-1.75) in 1996-97 to 2.88
56
57 (95% CI 1.64-5.05) in 2006-07 to 5.26 (95% CI 1.57-17.65) in 2016 (2016 estimates were only
58
59 based on Bissau data) (ratios of ratios based on Table 1).
60

Discussion

As we had hypothesized, the female/male HIV-1 prevalence ratio increased for the age group 15-34 years, as the proportion with smallpox vaccination scars decreased, whereas the female/male HIV-1 prevalence ratio remained unchanged for the age group ≥ 35 years, which had a steady smallpox vaccination coverage over the HIV-1 survey years.

Strengths and limitations

This study was based on information from large HIV surveys carried out over 20-30 years in two different settings, urban and rural Guinea-Bissau. As no central smallpox vaccination register exists in Guinea-Bissau, we used smallpox vaccination scars as a proxy for the smallpox vaccination coverage. We have previously shown that smallpox scars have a sensitivity of $>90\%$ in correctly identifying smallpox vaccinated individuals (no specificity measure available).⁽⁷⁾ Some BCG vaccination scars and accidental wounds may have been misclassified as smallpox vaccination scars, but misclassification is unlikely to be sex-differential. Potential variation in false-positive and false-negative rates of scar across surveys would likewise not be expected to be sex-differential. Participation in the HIV surveys varied only slightly across the survey years in Bissau, being 86% in 1987, 85% in 1996, 79% in 2006 and 83% in 2016; furthermore, the HIV prevalence in participants, who were easy to reach, was similar to the prevalence in those who were difficult to reach.^(4, 9) Hence, differential participation in different study years is unlikely to have caused selection bias.

The ecological design enabled us to investigate a potentially important hypothesis, but the results needs to be interpreted with caution since this design can be vulnerable to misinterpretations. By using the ratio of HIV-1 prevalence between sexes and within age groups, we could to some extent

1
2 disregard calendar time trends such as the spread, prophylaxis and treatment of HIV-1 affecting
3
4 both sexes and all age groups.
5
6
7
8
9

10 11 **Female/male HIV-1 prevalence trends in Sub-Saharan Africa**

12 Consistent with our finding, cross sectional surveys from Malawi,(11) Zambia,(12) and South
13
14 Africa(13) show that the birth cohorts who are too young to have been smallpox vaccinated have an
15
16 increased female/male HIV prevalence compared with older birth cohorts, who are likely to have
17
18 been smallpox vaccinated before the worldwide phase-out in 1980.
19
20
21
22

23
24
25 UNAIDS data for the female/male HIV-1 prevalence from 1985 to 2003 in Sub-Saharan Africa
26
27 shows that in the 15-49-year-old age group, the number of HIV-1 affected women began to increase
28
29 over the number of men during the early 1990s.(14) The female/male HIV-1 ratio increased at the
30
31 same time as the smallpox vaccination coverage decreased after 1980. A multi-country study using
32
33 repeated national representative demographic and health surveys on HIV prevalence in Sub-Saharan
34
35 Africa during the 2000s did not find an increasing female/male HIV prevalence ratio.(15) In
36
37 contrast to the HIV surveys from Guinea-Bissau, where there was a clear increase in the prevalence
38
39 of HIV-1, the reported HIV prevalence generally decreased between repeated surveys in other
40
41 regions of Africa.(15) The female/male HIV prevalence ratio may be influenced by multiple factors,
42
43 and the introduction of HIV treatment, which only took place in the late 2000s in Guinea-
44
45 Bissau,(16) may have blurred the female/male HIV prevalence ratio trends in the 2000s surveys
46
47 from Sub-Saharan Africa.
48
49
50
51
52
53
54

55 **Potential causes of sex differences in HIV-1**

56
57 The sex differences in the susceptibility to HIV-1 could theoretically be due to physiological,
58
59 hormonal or local microbial differences, and higher prevalence of sexually transmitted diseases
60

1 causing a higher male-to-female than female-to-male HIV transmission rate.(14) These
2
3 explanations would however not explain why young women in Sub-Saharan Africa did not have a
4
5 higher HIV-1 prevalence than men when the HIV epidemic started. Our results showed that women
6
7 and men in the age group <35 years had similar HIV-1 prevalence in 1987-1990 of 0.3% but while
8
9 men's HIV-1 prevalence did not increase much in the younger age group, potentially due to more
10
11 focus on availability and use of condoms over time, women's HIV-1 prevalence continued to
12
13 increase (Table 1). It may be that females' increased susceptibility were neutralized by the smallpox
14
15 vaccination and became expressed when smallpox vaccination was stopped.
16
17
18
19
20
21

22 Alternative explanations for the sex-age-time pattern may be sought in social and cultural changes
23
24 over time, including gender-power imbalances.(14) Analyses of sexual mixing patterns from South
25
26 Africa(13) suggest that since young women often have sexual relations with older men, then as the
27
28 prevalence of HIV-1 increases among older men the prevalence among young women will follow.
29
30 We have no specific data to assess possible changes over time in the frequency of sexual relations
31
32 across age groups. All ethnic groups in Guinea-Bissau has a taboo on intercourse while the mother
33
34 is breastfeeding for 1½-3 years, which may have created a permissive attitude towards extra-marital
35
36 sexual relationships (possible causing sexual relations between older men and younger women). We
37
38 have documented such taboo on intercourse while breastfeeding and a permissive attitude back to
39
40 the 1980s in Guinea-Bissau (17) so it is clearly not a new phenomenon. While it is possible that
41
42 behavioural patterns became increasingly permissive of extra-marital sexual relationships in a
43
44 setting with rapidly increasing urbanization, it seems unlikely that the same change would have
45
46 happened in a rural setting. We find it unlikely that the similar pattern of increasing female/male
47
48 HIV-1 prevalence ratio in both an urban and a rural setting can be explained merely by changes in
49
50 sexual behaviour patterns. During the study period, there may have been increased awareness and
51
52 availability of condoms, but this would likely have affected the risk of acquiring HIV equally in
53
54 both sexes or if anything diminished the risk in females relative to males.
55
56
57
58
59
60

1
2
3
4 The female/male HIV-1 prevalence ratio for the age group 15-34 seems to continue to increase in
5
6 Bissau with a ratio of 5.41 (95% CI 2.15-13.61) in 2016 compared with 2.34 (95% CI 1.35-4.04) in
7
8 2006, despite the prevalence of smallpox vaccination coverage for this age group was estimated to
9
10 change from 4% to 0%, suggests that other factors continue to affect the susceptibility of HIV-1
11
12 differently for men and women.
13
14
15
16
17

18 **Biological mechanisms**

19
20 The CCR5 is fundamental for establishing HIV-1 infections.(18) The CCR5-delta-32 deletion
21
22 confers resistance to HIV-1 by preventing the expression of the CCR5 receptor; this allele provides
23
24 almost complete resistance to HIV-1 in homozygous individuals.(18) A recent immunological study
25
26 found that cells from smallpox vaccinated individuals had up to 5-fold reduction in CCR-5 tropic
27
28 HIV-1 replication *in vitro*,(2) which supports a role of the smallpox vaccine in HIV-1 prevention
29
30 through heterologous immunity. A recent study did not show an association between smallpox
31
32 vaccination scar and CCR5 expression on the surface of peripheral T-lymphocytes among HIV
33
34 seronegative women old enough to have had a chance of being smallpox vaccinated;(19) this may
35
36 be due to delay between smallpox vaccination and immunological testing of more than four decades
37
38 or that the smallpox-unvaccinated control group had received another immunomodulator, the BCG
39
40 vaccine.(20)
41
42
43
44
45
46
47

48 In animal models, administrating smallpox vaccination via skin scarification has been demonstrated
49
50 to increase the immune response and survival compared with other modes of administration.(21)
51
52 Murine studies have shown that intradermal smallpox vaccination induced long-lived non-
53
54 recirculating CD8+ skin resident T-memory cells that resided within the entire skin and protected
55
56 against reinfection.(22) This indicates that vaccination can spread throughout the entire epithelial
57
58 surface to create a “shield” against infection.
59
60

1
2
3
4 Smallpox vaccine may also affect the innate immune system more broadly; in a very recent study,
5
6 human monocytes trained with smallpox vaccine showed significantly increased IL-6 and TNF- α
7
8 production to stimulation with non-related stimuli, compared to non-trained monocytes.(23)
9
10

11
12
13 Overall, there is some immunological evidence to support that smallpox vaccination can provide
14
15 cross-protection against HIV-1 infection. None of the above studies reported effects by sex, but it is
16
17 plausible that an epithelial protection might be particularly protective against vaginally acquired
18
19 HIV-1 infection.
20
21
22
23
24

25 **Conclusion**

26
27 Our hypothesis that the phase-out of smallpox vaccination may have increased the female/male
28
29 HIV-1 prevalence ratio was compatible with our results. This hypothesis needs further assessments
30
31 to determine if the relationship is causal, and we hope other research groups will test the hypothesis
32
33 and other potential explanations for the change in female-male HIV prevalence ratios over time in
34
35 individual-based data. If more support for the hypothesis that smallpox vaccine protected females
36
37 against HIV can be obtained, from epidemiological and immunological studies, it could provide
38
39 important information for HIV-1 vaccine research even though it may not be possible to reintroduce
40
41 the smallpox vaccine.
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Author contact information:

Andreas Rieckmann, Bandim Health Project, OPEN, Institute of Clinical Research, University of Southern Denmark/Odense University Hospital, Odense, Denmark. Research Center for Vitamins and Vaccines (CVIVA), Bandim Health Project, Statens Serum Institut, Artillerivej 5, DK-2300 Copenhagen S, Denmark and Section of Epidemiology, Department of Public Health, University of Copenhagen, Copenhagen, Denmark. anri@ssi.dk

Marie Villumsen, Center for Clinical Research and Prevention, Bispebjerg and Frederiksberg Hospital, Capital Region, Copenhagen, Denmark. marie.villumsen@regionh.dk

Bo Langhoff Hønge, Department of Clinical Immunology, Aarhus University Hospital, Denmark and Bandim Health Project, Indepth Network, Apartado 861, Bissau, Guinea-Bissau. bohonge@gmail.com

Signe Sørup, Bandim Health Project, OPEN, Institute of Clinical Research, University of Southern Denmark/Odense University Hospital, Odense, Denmark. Research Center for Vitamins and Vaccines (CVIVA), Bandim Health Project, Statens Serum Institut, Artillerivej 5, DK-2300 Copenhagen S, Denmark and Department of Clinical Epidemiology, Aarhus University, Olof Palmes Allé 43-45, DK-8200 Aarhus N, Denmark. SGS@ssi.dk

Amabélia Rodrigues, Bandim Health Project, Indepth Network, Apartado 861, Bissau, Guinea-Bissau. a.rodrigues@bandim.org

Zacarias José da Silva, National Institute of Public Health (INASA), CP 1013, Bissau, Guinea-Bissau. zacarias55@hotmail.com

Hilton Whittle, London School of Hygiene and Tropical Medicine, Keppel Street, London, UK, hcwhittle@yahoo.co.uk

Christine Stabell Benn, Bandim Health Project, OPEN, Institute of Clinical Research, University of Southern Denmark/Odense University Hospital, Odense, Denmark. Research Center for Vitamins and Vaccines (CVIVA), Bandim Health Project, Statens Serum Institut, Artillerivej 5, DK-2300

1
2 Copenhagen S, Denmark and OPEN, Odense University Hospital/Institute of Clinical Research,
3
4 University of Southern Denmark, Odense, Denmark. CB@ssi.dk
5

6 Peter Aaby, Bandim Health Project, Indepth Network, Apartado 861, Bissau, Guinea-Bissau.
7

8
9 p.aaby@bandim.org
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

References

1. Mayr A. Taking advantage of the positive side-effects of smallpox vaccination. *Journal of veterinary medicine B, Infectious diseases and veterinary public health*. 2004;51(5):199-201.
2. Weinstein RS, Weinstein MM, Alibek K, Bukrinsky MI, Brichacek B. Significantly reduced CCR5-tropic HIV-1 replication in vitro in cells from subjects previously immunized with Vaccinia Virus. *BMC immunology*. 2010;11:23.
3. Rieckmann A, Villumsen M, Jensen ML, Ravn H, Silva ZJd, Sørup S, et al. The effect of smallpox and BCG vaccination on the risk of HIV-1 infection in Guinea-Bissau and Denmark. *Open Forum Infect Dis*. 2017.
4. da Silva ZJ, Oliveira I, Andersen A, Dias F, Rodrigues A, Holmgren B, et al. Changes in prevalence and incidence of HIV-1, HIV-2 and dual infections in urban areas of Bissau, Guinea-Bissau: is HIV-2 disappearing? *Aids*. 2008;22(10):1195-202.
5. Tienen C, van der Loeff MS, Zaman SM, Vincent T, Sarge-Njie R, Peterson I, et al. Two distinct epidemics: the rise of HIV-1 and decline of HIV-2 infection between 1990 and 2007 in rural Guinea-Bissau. *Journal of acquired immune deficiency syndromes*. 2010;53(5):640-7.
6. Rieckmann A, Villumsen M, Jensen ML, Ravn H, da Silva ZJ, Sorup S, et al. The Effect of Smallpox and Bacillus Calmette-Guerin Vaccination on the Risk of Human Immunodeficiency Virus-1 Infection in Guinea-Bissau and Denmark. *Open forum infectious diseases*. 2017;4(3):ofx130.
7. Aaby P, Gustafson P, Roth A, Rodrigues A, Fernandes M, Sodemann M, et al. Vaccinia scars associated with better survival for adults. An observational study from Guinea-Bissau. *Vaccine*. 2006;24(29-30):5718-25.
8. Jensen ML, Dave S, Schim van der Loeff M, da Costa C, Vincent T, Leligdowicz A, et al. Vaccinia scars associated with improved survival among adults in rural Guinea-Bissau. *PloS one*. 2006;1:e101.
9. Olesen JS, Jespersen S, da Silva ZJ, Rodrigues A, Erikstrup C, Aaby P, et al. HIV-2 continues to decrease, whereas HIV-1 is stabilizing in Guinea-Bissau. *Aids*. 2018;32(9):1193-8.
10. Månsson F. HIV-1, HIV-2, and other Sexually Transmitted Infections in GuineaBissau, West Africa. Lund University. 2012.
11. Maman D, Chilima B, Masiku C, Ayouba A, Masson S, Szumilin E, et al. Closer to 90-90-90. The cascade of care after 10 years of ART scale-up in rural Malawi: a population study. *Journal of the International AIDS Society*. 2016;19(1):20673.
12. Chanda-Kapata P, Kapata N, Klinkenberg E, William N, Mazyanga L, Musukwa K, et al. The adult prevalence of HIV in Zambia: results from a population based mobile testing survey conducted in 2013-2014. *AIDS research and therapy*. 2016;13:4.
13. de Oliveira T, Kharsany AB, Graf T, Cawood C, Khanyile D, Grobler A, et al. Transmission networks and risk of HIV infection in KwaZulu-Natal, South Africa: a community-wide phylogenetic study. *The lancet HIV*. 2017;4(1):e41-e50.
14. Quinn TC, Overbaugh J. HIV/AIDS in women: an expanding epidemic. *Science*. 2005;308(5728):1582-3.
15. Hegdahl HK, Fylkesnes KM, Sandoy IF. Sex differences in HIV prevalence persist over time: Evidence from 18 countries in Sub-Saharan Africa. *PloS one*. 2016;11(2):e0148502.
16. Jespersen S, Honge BL, Oliveira I, Medina C, da Silva Te D, Correia FG, et al. Challenges facing HIV treatment in Guinea-Bissau: the benefits of international research collaborations. *Bulletin of the World Health Organization*. 2014;92(12):909-14.
17. Høgsborg M, Aaby P. Sexual relations, use of condoms and perceptions of AIDS in an urban area of Guinea-Bissau with a high prevalence of HIV-2. *Sexual behaviour and networking: anthropological and socio-cultural studies on the transmission of HIV*, edited by Tim Dyson Liege, Belgium, Editions Derouaux-Ordina, [1992] 203-31. 1992.
18. Dean M, Carrington M, Winkler C, Huttley GA, Smith MW, Allikmets R, et al. Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. *Hemophilia Growth and Development Study, Multicenter AIDS Cohort Study, Multicenter Hemophilia Cohort Study, San Francisco City Cohort, ALIVE Study*. *Science*. 1996;273(5283):1856-62.
19. Beck KB, Honge BL, Olesen JS, Petersen MS, Jespersen S, Wejse C, et al. Long-term effects of smallpox vaccination on expression of the HIV-1 co-receptor CCR5 in women. *PloS one*. 2018;13(11):e0207259.
20. Netea MG, Joosten LA, Latz E, Mills KH, Natoli G, Stunnenberg HG, et al. Trained immunity: A program of innate immune memory in health and disease. *Science*. 2016;352(6284):aaf1098.
21. Rice AD, Adams MM, Lindsey SF, Swetnam DM, Manning BR, Smith AJ, et al. Protective properties of vaccinia virus-based vaccines: skin scarification promotes a nonspecific immune response that protects against orthopoxvirus disease. *Journal of virology*. 2014;88(14):7753-63.
22. Jiang X, Clark RA, Liu L, Wagers AJ, Fuhlbrigge RC, Kupper TS. Skin infection generates non-migratory memory CD8+ T(RM) cells providing global skin immunity. *Nature*. 2012;483(7388):227-31.

1
2 23. Blok BA, Jensen KJ, Aaby P, Fomsgaard A, van Crevel R, Benn CS, et al. Opposite effects of Vaccinia
3 and modified Vaccinia Ankara on trained immunity. *European journal of clinical microbiology & infectious diseases* :
4 official publication of the European Society of Clinical Microbiology. 2019;38(3):449-56.
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

1
2 **Figure 1. Estimated smallpox vaccination scar prevalence in different age groups for each**
3
4 **sequential HIV survey**
5

6
7
8 Insert figure 1
9

10 Based on data from Bissau, Guinea-Bissau, previously published.(6) The estimation for the age
11 group ≥ 35 in 2016 was changed to ≥ 45 to ensure a steady smallpox vaccination coverage.
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46

1 **Table 1. The female/male HIV-1 prevalence ratio (PR) by age group, survey year and study site**

Age group	15-34 years (decreasing smallpox vaccination rates in later years; see Figure 1)				≥35 years (steady smallpox vaccination rate over time; see Figure 1)			
	Estimated smallpox coverage	HIV-1 prevalence (%)		Female/male PR (95% CI)	Estimated smallpox coverage	HIV-1 prevalence (%)		Female/male PR (95% CI)
Study site and survey year		Female	Male			Female	Male	
Caió								
1990	62%	0.3% (3/861)	0.4% (2/541)	0.94 (0.16-5.62)	72%	0.8% (7/907)	0.4% (2/461)	1.78 (0.37-5.53)
1997	27%	1.8% (17/958)	1.9% (14/738)	0.94 (0.46-1.89)	77%	4.4% (41/943)	2.8% (13/471)	1.58 (0.85-2.91)
2007	4%	3.2% (28/885)	1.5% (11/742)	2.13 (1.07-4.26)	71%	4.8% (41/850)	6.0% (25/418)	0.81 (0.50-1.31)
Bissau								
1987	62%	0% (0/243)	0% (0/197)	NA	72%	0% (0/110)	0% (0/99)	NA
1996	27%	2.2% (19/881)	1.5% (10/680)	1.47 (0.69-3.13)	77%	3.3% (13/394)	3.5% (12/346)	0.95 (0.44-2.06)
2006	4%	5.3% (56/1056)	2.3% (16/705)	2.34 (1.35-4.04)	71%	5.4% (25/466)	6.5% (21/321)	0.82 (0.47-1.44)
2016	0%	4.2% (41/983)	0.8% (5/648)	5.41 (2.15-13.61)	66%	5.2% (13/252)**	5.0% (11/219)**	1.03 (0.23-2.25)*
Combined								
1987-90	62%	0.3% (3/1104)	0.3% (2/738)	1.00 (0.17-5.99)	72%	0.7% (7/1017)	0.4% (2/560)	1.93 (0.40-2.25)
1996-97	27%	2.0% (36/1839)	1.7% (24/1418)	1.16 (0.69-1.93)	77%	4.0% (54/1337)	3.1% (25/817)	1.32 (0.83-2.10)

2006-07	4%	4.3% (84/1941)	1.9% (27/1447)	2.32 (1.51-3.56)	71%	5.0% (66/1316)	6.2% (46/739)	0.81 (0.56-1.16)
---------	----	-------------------	-------------------	------------------	-----	-------------------	------------------	------------------

- 2 Data are extracted from (4, 5, 9).
- 3 * This estimate is only based on information from Bissau.
- 4 ** The estimation for the age group ≥ 35 in 2016 was changed to ≥ 45 to ensure a steady smallpox vaccination coverage.

For peer review only

1
2
3 **Figure 2. Female/male HIV-1 prevalence ratios, Bissau and Caió**
4

5
6 Insert figure 2
7

8 Visualisations of estimates from Table 1. Circles and triangles represent point estimates and lines
9 represent the 95% confidence intervals. The estimation for the age group ≥ 35 in 2016 was only
10 from Bissau and was changed to ≥ 45 to ensure a steady smallpox vaccination coverage.
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

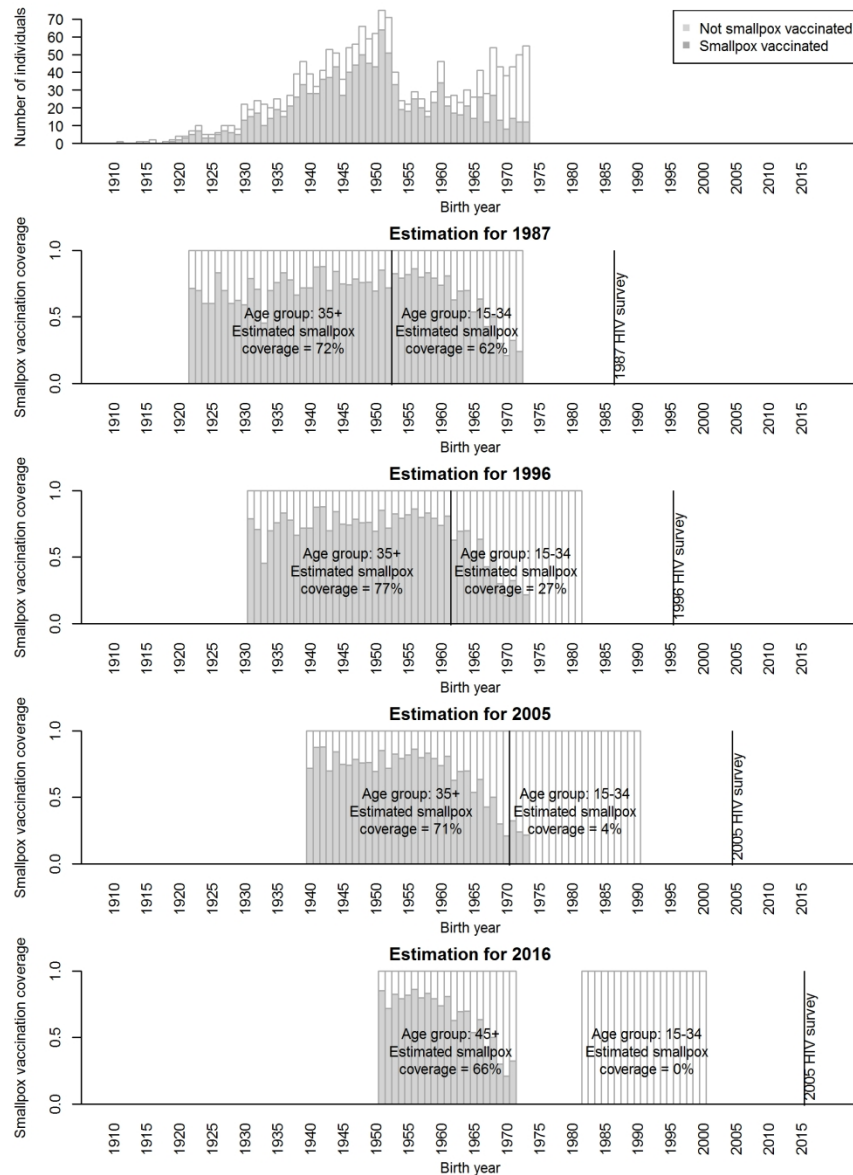


Figure 1. Estimated smallpox vaccination scar prevalence in different age groups for each sequential HIV survey.

Based on data from Bissau, Guinea-Bissau, previously published.(6) The estimation for the age group ≥ 35 in 2016 was changed to ≥ 45 to ensure a steady smallpox vaccination coverage

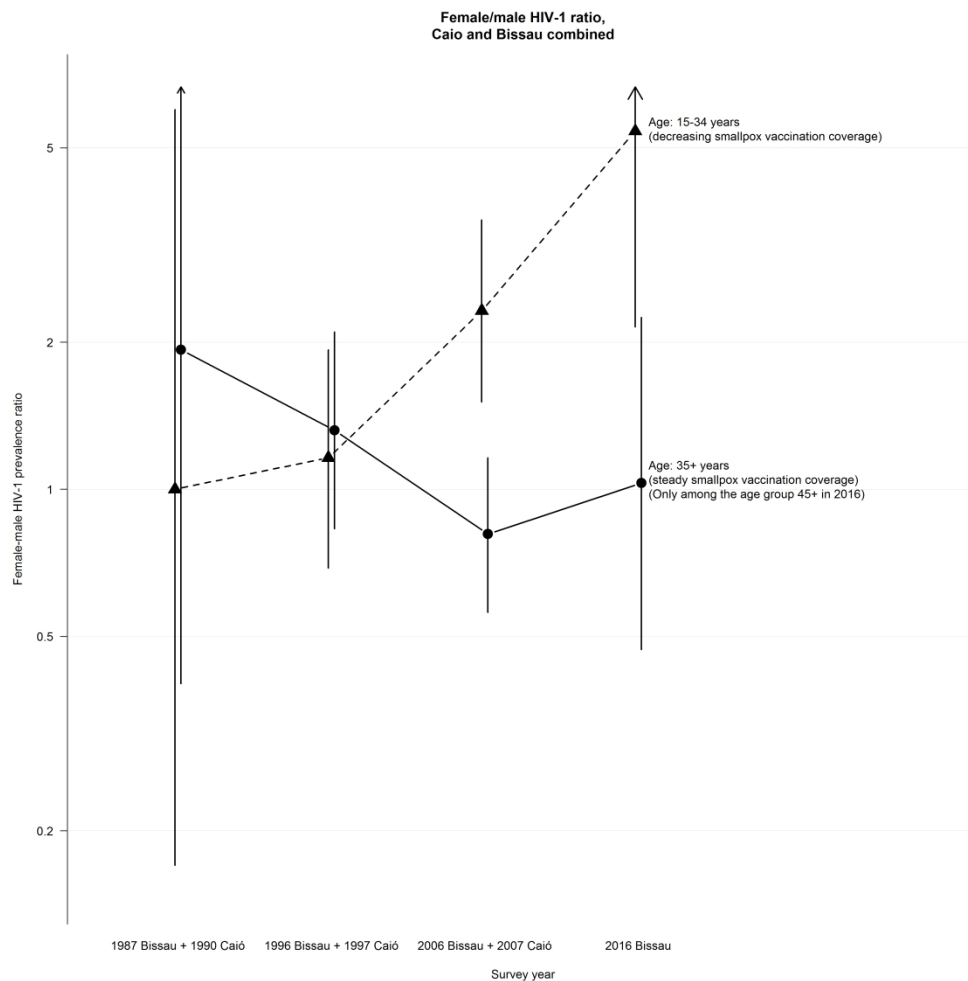
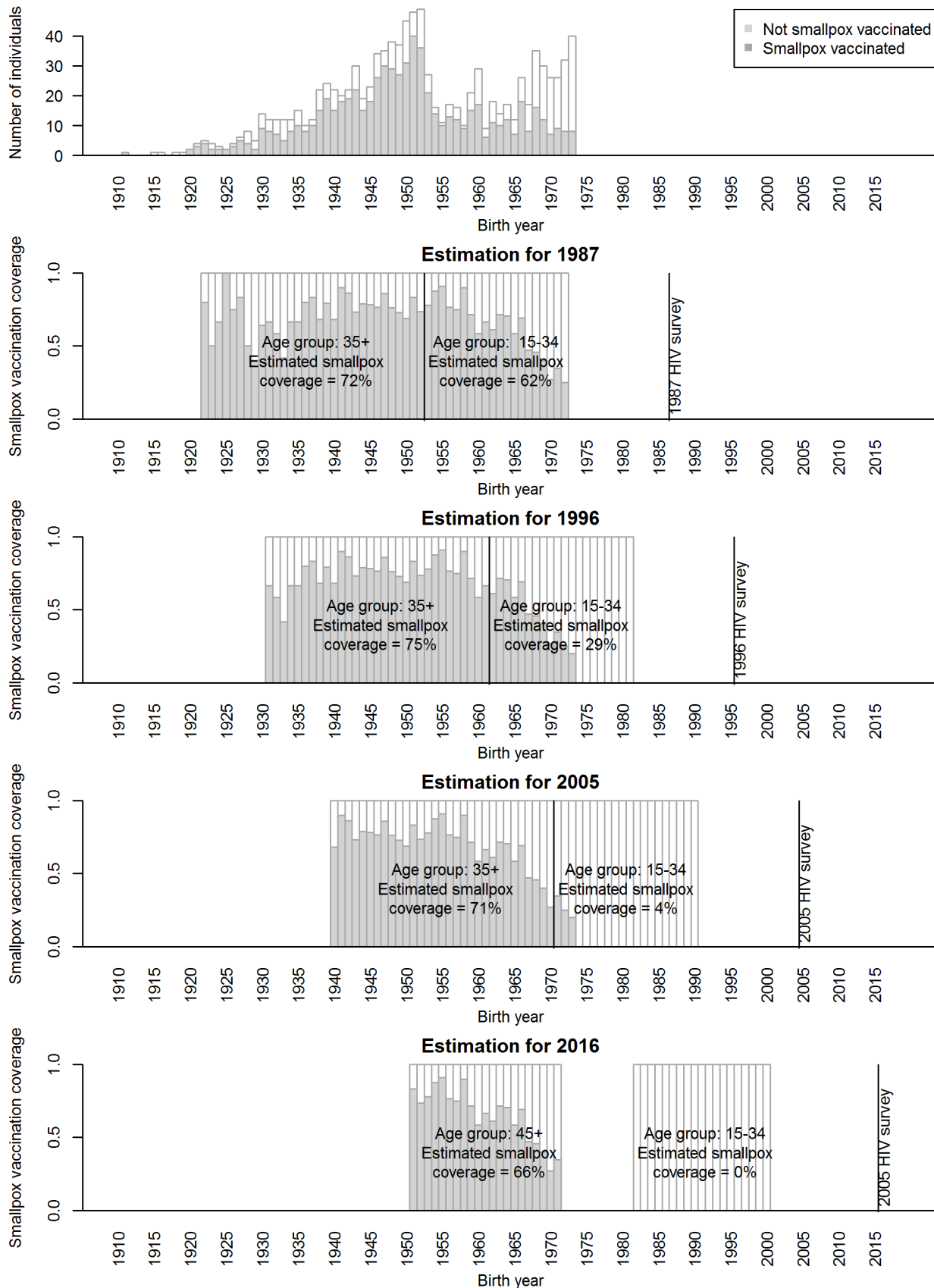


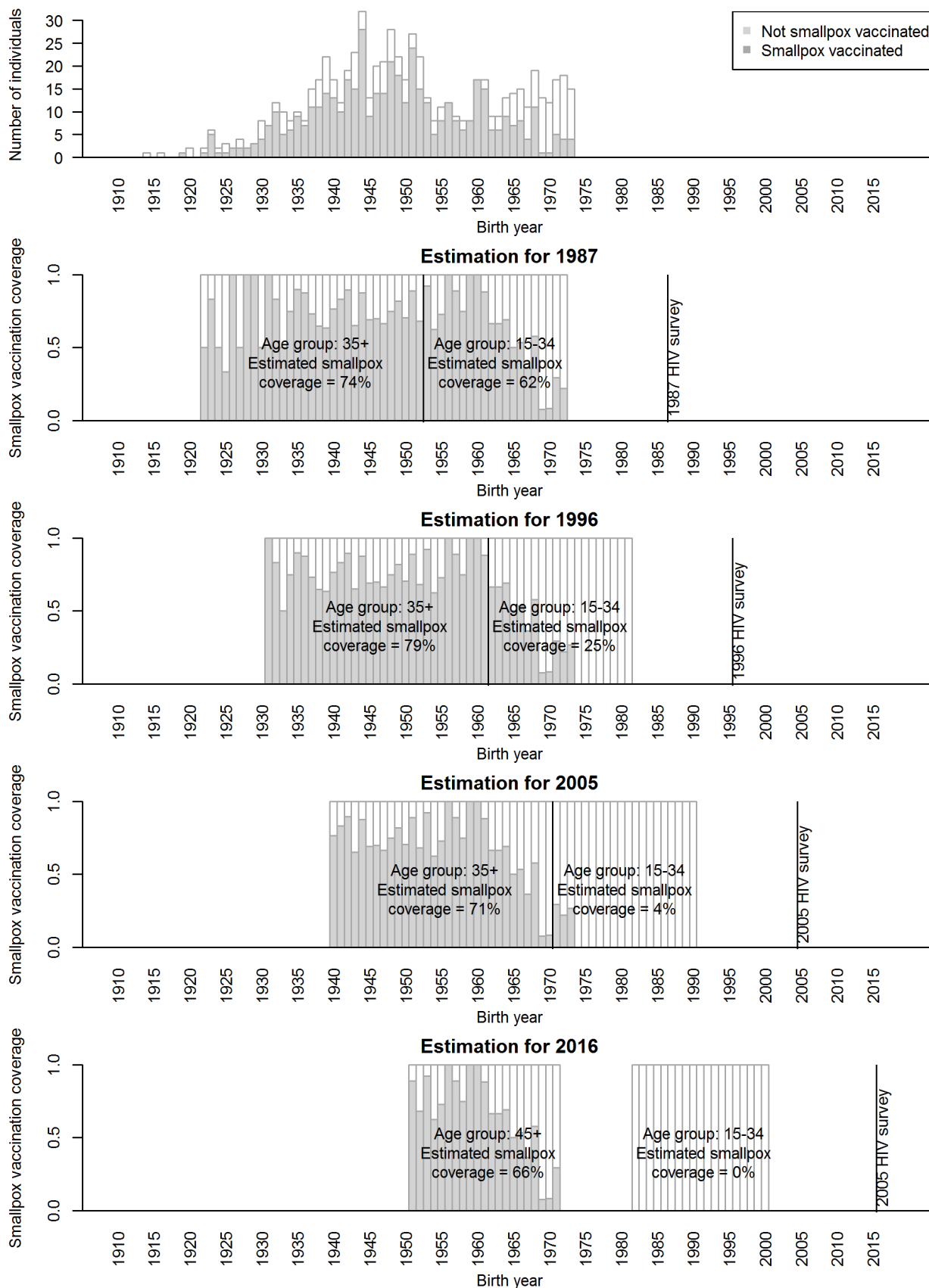
Figure 2. Female/male HIV-1 prevalence ratios, Bissau and Caió.

Visualisations of estimates from Table 1. Circles and triangles represent point estimates and lines represent the 95% confidence intervals. The estimation for the age group ≥ 35 in 2016 was only from Bissau and was changed to ≥ 45 to ensure a steady smallpox vaccination coverage.

Supplementary figure 1. Estimated smallpox vaccination scar prevalence in different age groups for each sequential HIV survey among women



Supplementary figure 2. Estimated smallpox vaccination scar prevalence in different age groups for each sequential HIV survey among men



1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60STROBE Statement—Checklist of items that should be included in reports of *cross-sectional studies*

	Item No	Recommendation	Responses	Page
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	✓	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	✓	3
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	✓	5
Objectives	3	State specific objectives, including any prespecified hypotheses	✓	5
Methods				
Study design	4	Present key elements of study design early in the paper	✓	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	✓	5-6
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants	✓	5-7
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	✓	6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	✓	5-8
Bias	9	Describe any efforts to address potential sources of bias	✓ (these are discussed)	7-8
Study size	10	Explain how the study size was arrived at	✓	5-6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	✓	5-8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	✓	8
		(b) Describe any methods used to examine subgroups and interactions	✓	8
		(c) Explain how missing data were addressed	NA, as the study builds upon published data	6
		(d) If applicable, describe analytical methods taking account of sampling strategy	NA, as the study builds upon published data	6
		(e) Describe any sensitivity analyses	The ecological data allowed	NA

			for one man analysis of two sites	
Results				
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	✓ (this is an ecological study)	Table 1
		(b) Give reasons for non-participation at each stage	✓ (this is an ecological study)	NA
		(c) Consider use of a flow diagram	✓ (this is an ecological study, and we chose not to include a flow chart)	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	✓	5-8
		(b) Indicate number of participants with missing data for each variable of interest	NA, as the study builds upon published data	NA
Outcome data	15*	Report numbers of outcome events or summary measures	✓	Table 1
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	✓ (the comparison group above 35 years function an adjustment of calendar time)	Table 1
		(b) Report category boundaries when continuous variables were categorized	✓ Age was dichotomised	5-8
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	✓ (we report relative risks and absolute values)	8-9
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	✓ (all analyses are presented)	8-9
Discussion				
Key results	18	Summarise key results with reference to study objectives	✓	10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	✓	10-11

1				
2	Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	✓ 11-14
3				
4				
5				
6	Generalisability	21	Discuss the generalisability (external validity) of the study results	✓ 11-12
7				
8	Other information			
9				
10	Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	✓ 2
11				
12				
13				
14				

15 *Give information separately for exposed and unexposed groups.

16
17
18 **Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60