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Seroprevalence of AAV-1, -8, and -9 in People Living With and Without HIV in South Africa and Zimbabwe

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BACKGROUND

- Adeno-associated virus (AAV)-vectored delivery of monoclonal (m) HIV-specific broadly (b) neutralizing (n) antibodies (Abs) holds promise for treating HIV infection.
- Successful AAV/bnAb transduction of long-lived cells, such as myocytes, can result in continuous bnAb expression for years, possibly decades.
- A key barrier to the clinical use of AAV/bnAb vectors is the high prevalence of pre-existing anti-AAV nAbs in humans. However, nAb titers <1:160 allows intramuscular (IM) AAV transduction in AAV+ primates (PMID: 27817961).
- It is critical to determine the seroprevalence of muscle-tropic AAV capsids in areas with high incidence of HIV, so that people living with HIV (PLWH) who are seronegative for these AAV capsids can potentially benefit from AAV/bnAb therapies without any additional intervention.
- AAV epidemiology is well documented in developed countries but nearly absent for sub-Saharan Africa, home of nearly two thirds of all the PLWH.
- To address this deficit, we partnered with the HVTN (HIV Vaccine Trials Network) and ACTG (AIDS Clinical Trials Group) to establish the prevalence of anti-AAV nAbs in South Africa and Zimbabwe.
- We selected AAV-9 for an in-depth titer analysis because of its superior performance to AAV-1 and -8 in promoting persistent mAb expression in nonhuman primates following IM administration (PMID 37153616).

METHODS

- HEK293T cells and luciferase-expressing AAV vectors were used to screen sera from 300 adult people living without HIV (PLWOH) and 277 PLWH for nAbs against AAV-1, -8, and -9.
- Serum samples were first diluted 1:20 and mixed with each AAV/Fluc vector to achieve a level of transduction that yielded a luciferase signal at least ten times above background.
- The midpoint titers of anti-AAV-9 nAbs in the subset of samples that exhibited ≥ 50% neutralization at 1:20 dilution were then determined by diluting 3-fold up to 1:540 and using a non-linear regression model.

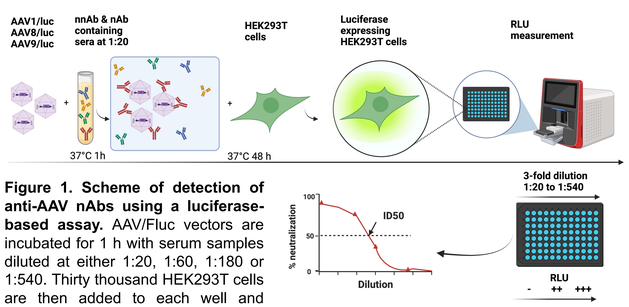


Figure 1. Scheme of detection of anti-AAV nAbs using a luciferase-based assay. AAV/Fluc vectors are incubated for 1 h with serum samples diluted at either 1:20, 1:60, 1:180 or 1:540. Thirty thousand HEK293T cells are then added to each well and incubated for 48 h. Relative light units (RLUs) are measured in luminometer and the % neutralization is calculated. ID_{50} titer is determined using logistical regression.

71% of PLWH and 79% of PLWOH in South Africa and Zimbabwe display levels of preexisting anti-AAV-9 nAbs that could make them eligible to participate in clinical trials of IM-delivered AAV-9 vectors

RESULTS

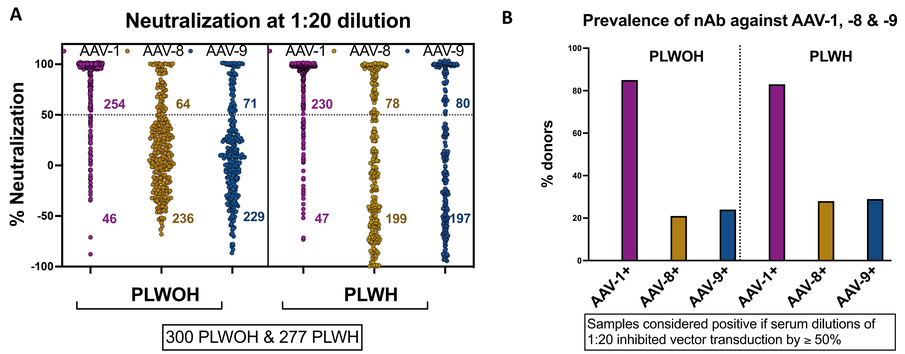


Figure 2. Seroprevalence of AAV-1, -8, and -9 in PLWOH) and PLWH in Sub-Saharan Africa. (A) 577 human samples (300 from PLWOH and 277 from PLWH) were screened for preexisting neutralizing antibodies against the indicated AAV serotype using a luciferase-based neutralization assay. Each dot represents an individual sample. **(B)** % of samples by cohort considered positive or negative for nAbs against each capsid. Samples that inhibited vector transduction ≥ 50% at 1:20 dilution were deemed positive.

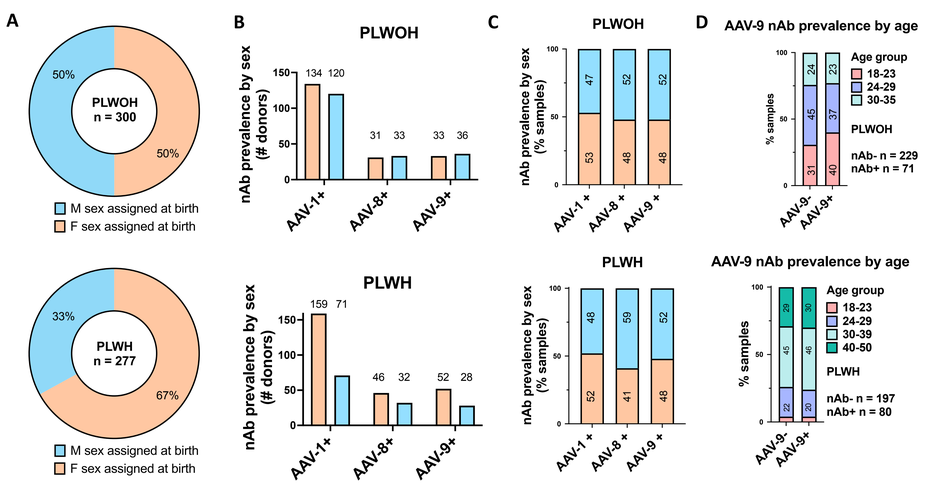


Figure 3. Stratification by sex of AAV-1 and AAV-8, by sex and age of AAV-9 seroprevalence among PLWOH and PLWH. (A) 300 samples from PLWOH (balanced by sex) and 277 from PLWH (67% female samples and 33% male samples) were screened for nAbs against AAV-1, -8, and -9. **(B) (C) (D)** No notable difference in seroprevalence was observed between females and males and between different age groups in both cohorts (PLWH cohort was normalized by F:M ratio).

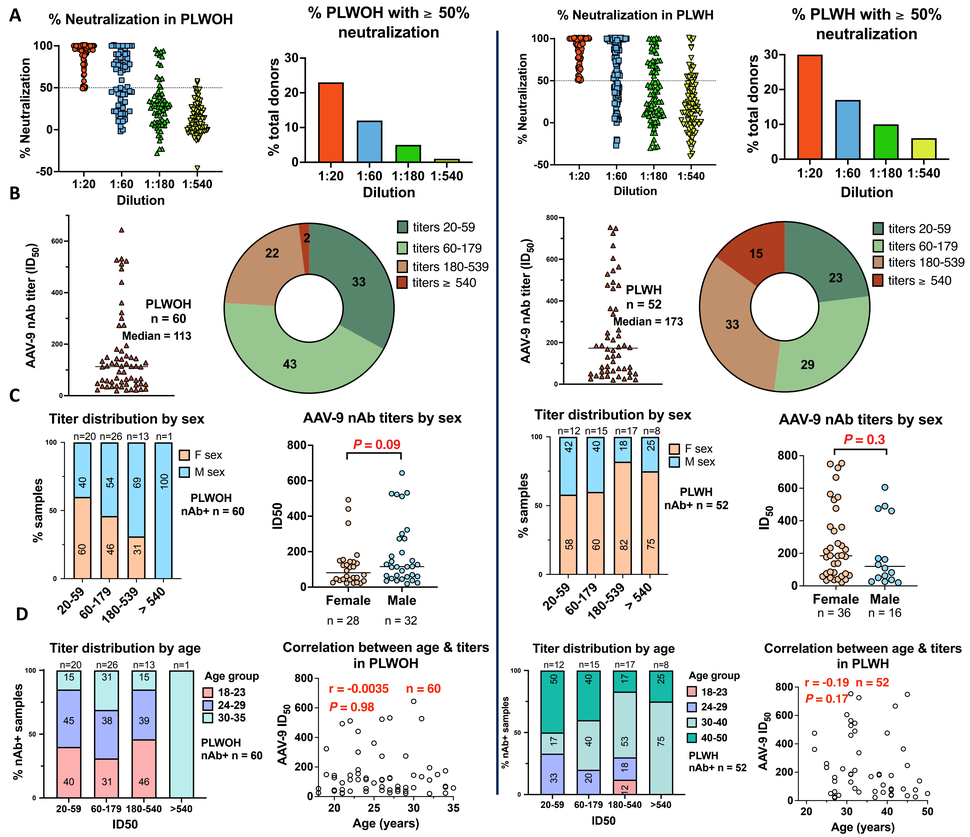


Figure 4. Seroprevalence and magnitude of titers against AAV-9 among PLWOH and PLWH (A) % neutralization of individual samples and % of total samples with ≥ 50% neutralization at different dilutions **(B).** ID_{50} values from 60 PLWOH and 52 PLWH samples were determined, median values of 113 and 173 in subsets of PLWOH and PLWH, respectively. **(C)** Stratification by sex and non-parametric t-test in both cohorts. **(D)** Stratification by age and Spearman correlation between age and titers.

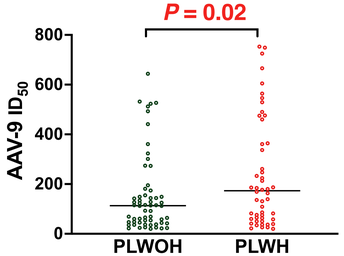


Figure 5. Comparison of AAV-9 nAb titers in PLWOH and PLWH. ID_{50} values from 60 PLWOH and 52 PLWH samples were determined by non-linear regression. Each dot represents an individual ID_{50} value. Mann-Whitney test analysis revealed that PLWH have significantly higher titers than PLWOH. Lines represent median values.

CONCLUSIONS

- 71% of PLWH and 79% of PLWOH in South Africa and Zimbabwe display preexisting anti-AAV9 nAbs titers of < 1:20.
- Among the AAV-9 nAb+ samples at 1:20, 52% of PLWH and 76% of PLWOH exhibit titers <180, a level that could make them eligible to participate in clinical trials of IM-delivered AAV9 vectors.
- No significant difference in seroprevalence and magnitude of AAV-9 nAb titers exists between males and females.
- Anti-AAV-9 nAb titers do not correlate with age.
- PLWH exhibit significantly higher titers of anti-AAV-9 nAbs than those in PLWOH.

ADDITIONAL KEY INFORMATION

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