As the end game of the polio eradication initiative approaches, it becomes more and more important to identify and assess the risk posed by potential reservoirs of neurovirulent poliovirus to prevent reemergence of the disease, poliomyelitis. This manuscript deals with the question of whether very young children who were infected with HIV could serve as one such reservoir through prolonged excretion of continually evolving, live polio vaccine that has the potential to revert to neurovirulent virus. Young orphaned children with antibodies to HIV were enrolled in this study. All were housed at the same institution. Stool samples were taken at intervals after the infants received OPV as part of a routine vaccination program and/or after NIDs. They were then analyzed for the presence of different serotypes of poliovirus to determine the duration of excretion. VP1 sequences of the polioviruses that were isolated were determined and used to confirm the duration of excretion by comparison with empirically determined evolutionary rates for polioviruses. Any non-polio enterovirus that were isolated from each stool sample were also serotyped. The anti-HIV antibody status of the infants was re-assessed to determine which infants were infected with HIV and which were misdiagnosed as HIV positive due to the presence of residual maternal anti-HIV antibodies. The latter individuals were then designated as controls.

General comments.

1. The question posed by the authors is well defined.
2. The methods are appropriate and well described.
3. The data is sound, but difficult to follow [see specific comments below]. The sample size is sufficient for this manuscript, however as the authors point out, the sample size is small and should be followed up by larger studies. The prospective nature and availability of serial specimens is one of the strong points
of this paper.

4. There is no indication that the sequence data was deposited in the GenBank/EMBL/DDBJ sequence databases. This must be done. Otherwise, the manuscript adheres to all relevant standards for reporting and data deposition.

5. The discussion and conclusions are balanced and adequately supported by data [See specific comments below].

6. The limits of the work are in general clearly stated (see specific comments below).

7. The authors acknowledge published and unpublished work that is the basis for this report [See specific comments].

8. The title is an accurate, neutral description of the findings in the paper and is acceptable. The authors might consider changing it to better reflect their conclusion that these HIV-infected children still maintain the ability to clear enteroviral infections. The abstract accurately conveys the results.

9. The manuscript is an important contribution and should be accepted for publication after specific revision [see specific comments below].

Specific comments. (Listed by page (P) and line (L) number or range, i.e., P4L10-12)

Discretionary Revisions

1. P4L11-18: Include some indication that most or all of the identified long time persistent poliovirus excretors have been B-cell deficient individuals whereas there would be little or no effect on B cells in the HIV infected children described in this report.

2. P4L21 Khetsuriani et al refer to enterovirus excretion in one child described in reference [12] but overlook the report of poliovirus in stools from 2 other children obtained at least 5-9 months after OPV vaccination. [Note: In ref 12 there is no indication that subsequent stool samples were polio-negative and no VP1 sequence data to help distinguish between prolonged excretion or a more recent exposure to OPV from a more recently vaccinated child.]

3. P8L4-5 The authors present a clear footnote explaining the different levels of immunosuppression. A similar concise explanation for category A and B of HIV-disease symptoms would be equally helpful for readers.

4. P8L8-9 Is it known what the children died from? More importantly, if they were not followed up, how was it determined whether they were HIV positive or “false positive” controls.

5. P12L19-22 Suggestion: Start a new paragraph with “All sequenced …. And include it as one paragraph with the following sentence “The degree of …”

6. P14L7-10 The orphanage employed a high standard of care and nutrition (P16L6) and this will most likely provide a hygienic barrier to host-to-host
transmission. For example no other patients excreted virus related to the diverged viruses of patient A. Another possibility is that the “switch” in the patients was equivalent to the intermittent shedding described for an immunodeficient individual described in a manuscript co-authored by some of the current authors (Reference 7 of this paper).

Minor Essential Revisions

1. P4L3: add “wild” [“Following wild polio …] since eradication implies elimination of wild and vaccine derived polio. Also remove “only” since there are other ways for reemergence (accidental release from vaccine storage or production facilities, archived stool samples collected during poliomyelitis endemic times that were overlooked or mislabeled during the containment arm of the eradication initiative, bioterror, etc).

2. P4L8 and P6L9 References #3 and #22 appear to be switched.

3. P6L9-13 Assigning the date of stool sample relative to the last date of OPV exposure might underestimate the time of excretion if persistence starts with a prior exposure. See specific request for an additional table below. This might be relevant for participant A where accumulated nucleotide substitutions are consistent with longer replication times than 87 days.

4. P9 2nd paragraph: The data presented is confusing. Inclusion of the table described below would clarify this information.

5. P10L10-12 Were the children excluded from the whole study or just from “this” duration determination part. Why were children excluded if only one sample was polio positive? (see also comment #6 above). Also why were routine administrations excluded but not NIDs (P10L16)? The most accurate times for exposure are from documented administration at the orphanage.

6. P10L20-21 When were these 3 doses administered (see request for table above). The infection could have been initiated from one of these exposures.

7. P10L22-P11L7 When was this child last exposed to OPV? The days listed are just days post-enrollment, not post-exposure. It is only stated that she arrived after the NIDs. Did she get the OPV in the orphanage as part of routine immunization when admitted or was she exposed to routine vaccination before admission? Her age in relation to the immunization program in Kenya would provide some indication. The finding of more than one serotype suggests that she was immunized. I believe that the answer is given in P14L11-16. If so, make the connection clearer. (The table would again make this clear).

8. P13L1-3. This child (142 day excretor) also excreted type 1 poliovirus. Please make clear the % divergences of the type 1 isolates from this child and represent them in the phylogenetic tree. [Again the table would help to know if these are already on the tree].

9. P13L5-8. The <6 months of persistence is based on the date of the last dose
of OPV. You need to qualify that if infection persisted from a previous exposure, these excretion times would be somewhat longer, however in all cases the polio and NPEV were cleared. This is particularly relevant for patient A.

10. P14L21 Patient A is not a prolonged shedder relative to the last dose but perhaps to the previous dose since the % divergence is not consistent with the former time interval. Also the time clock that is used here (and that the authors previously contributed significantly to its establishment) is primarily based on information from the VP1 and P1 and the rate would be decreased when including such areas as the 5'UTR that has a much lower rate due to structural restrictions. In any case the final numbers are still high and could be consistent with persistence form an earlier exposure.

11. P24 Table 1; last footnote Suggest change to “.. significant differences between HIV positive and negative groups. Since no differentiation is based on the category of disease or level of immunosuppression.

12. P14L4-8 The interval between exposure and positive samples is more important than the interval between positive samples. Data for the exact date of exposure is not available for some patients (see needed table below). It is not correct to say that none of the children shed divergent strains; more correct to say none except patient A since divergence reached approximately 1%.

Major Compulsory Revisions

1. p8L10-16 and throughout the rest of the paper. Required revision: It is difficult to follow who received what when. To help the reader understand the results and follow the result of individual children in this report, authors must add a table listing the participants in order by age for the HIV-infected children followed by a similar ranking of non-infected children. Then throughout the manuscript and in the figure refer to the specific children by using their identification letter from Column A.

Suggestion for table:

| Column A | Participant identification letter and age. |
| Column B: OPV exposures (routine (unknown, before, during enrollment) and NIDs) with footnote of dates of both NIDs and a reminder of recommended routine vaccination schedules for children in Kenya. |
| Columns C-G: (with as many repeats below columns C-G as necessary - one entry for each stool sample from that patient) individually listing all stool samples and contents. Information should include the number of days post last exposure, the serotypes of viruses isolated, and the % divergence of VP1 for polioviruses. Since information is online, the text might even be color-coded to reflect the virological information (negative, polio, NPEV, other). Example of 2 possible entries for the patient on P11L4 (the % divergence is arbitrary): |
| 87 115 |
PV3 PV3
0.01% 0.02%

The information about the OPV history for each child in column B is important. For example P12L1, 1% divergence corresponds to approximately 1 year of evolution. When was this child exposed to previous NIDs or routine vaccination? When was the child brought into orphanage? Since within 3 months, the child had significant vp1 divergence that increased 3 fold during that time with a complete genomic divergence of 2 fold overall. If possible include the interval in days between the last OPV and the previous OPV for this patient. It is not necessary for the other patients since VP1 sequence variability is low.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests.