Statistical Analysis Plan:

Oral polio vaccine as potential protection against COVID-19 A cluster-randomised trial in Guinea-Bissau

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1 General analysis principles

1.1 Participant population

Bandim Health Project monitors the health and survival of a population of approximately 100,000 individuals through a Health and Demographic Surveillance System (HDSS) implemented across six suburbs (Bandim-I, Bandim-II, Cuntum-I, Cuntum-II, Belem and Mindara) of Bissau. For the present trial, we visited residents aged >50 years living in five of the six suburbs in the HDSS to invite them to participate. People were invited to participate, if they did not have acute current infection, signs of immune suppression (≥2 pneumonias in the past year, oral candidiasis, fungal skin infections) or previous confirmed COVID-19. Furthermore, individuals with paralysis or past paralyses suspected to be caused by past thrombo-embolic complications were not offered enrolment due to the risk of recurrent events and paralysis, which could be confused with paralysis due to vaccination with oral polio vaccine (OPV). All analyses will be conducted on the per-protocol population.

1.2 Randomisation

All houses in five urban study area districts (Bandim-I, Bandim-II, Cuntum-I, Cuntum-II and Belem) were randomised to intervention or control group stratified by zone (Bandim-I: zones 1-9, Bandim-II: zones 1-8, Cuntum-I: zones 1-9, Cuntum-II: zones 2-4, and Belem zones A-E).

1.3 Baseline Interview

Prior to enrolment a list of potentially eligible people was extracted from the HDSS databases. At the enrolment visit, information on sex and age was confirmed, and all participants were interviewed on chronic diseases, symptoms on the day of enrolment, medicine intake in the past month and admissions within the past 3 months. We aimed to have minimal physical contact. Therefore, weight was assessed using a visual scoring system, based on the field assistant's perception of the participant's bodily appearance, scoring weight from 1-10[1]. The presence of a BCG and/or smallpox vaccination scar was also assessed by visual examination.

1.4 Follow-up

Participants were followed up through telephone calls and registration of consultations at two health centres in the study area where they could seek consultations free of charge. Furthermore, all individuals are also followed through the HDSS of Bandim Health Project.

1.4.1 Telephone follow-up

We called all study participants every 4 weeks between enrolment and 6 months of follow-up to collect information on health outcomes. In some cases, information was provided by a relative in the same household. For all trial participants, we asked whether they, since enrolment (for the 1st telephone interview call) or since the last call, had experienced any of the following symptoms:

> Common cold, Cough, Fever, Breath-lessness, Vomiting, Diarrhoea, Loss of sense of smell, Loss of sense of taste, Headache, Sore throat, Body aches, Extreme tiredness, Other symptoms (If yes: which?)

For "yes" to either of these symptoms, further questions on timing were asked (today, within the last week, 1-<2 weeks ago, 2-<3 weeks ago, 3 or more weeks ago)

Information was also collected on:

Use of other medicine than habitual, COVID-test performed (and result thereof), Weight loss during the interval

Finally, all participants were asked if they had sought consultation or been admitted to hospital during the interval. If yes, they were asked when, where, reason for consultation/admission and whether they had received a diagnosis.

1.4.2 Passive case detection

All participants were informed that they could seek consultation free of charge at two health centres in the study area. Following a consultation at the health centre, information on date of consultation, symptoms, diagnostic tests, and prescribed treatment were collected from the health centres consultation books.

1.5 Outcomes

All outcomes are assessed within the follow-up period of 6 months.

1.5.1. Primary outcome

The primary outcome is a composite outcome of the first of death, hospitalisation for infection and/or a recorded consultation for infection at the health centre. Thus, the information can come from either the telephone follow-up, the health centre data or the HDSS.

1.5.2. Secondary outcomes

The secondary outcomes are other measures of infectious disease morbidity (prespecified: a and b, specified after trial initiation: f) and the three separate components of the composite outcome (c, d, and e).

a) Episodes with self-reported infectious disease morbidity: Through the telephone follow-up any reported episode which includes Common cold, Cough, Fever, Breath-lessness, Vomiting, Diarrhoea, Sore throat, or Body aches will be counted as an infectious disease episode. Following an infection, the person will re-enter the at-risk-population 2 weeks later.

b) Episodes with self-reported infectious disease morbidity suspected to be caused by COVID-19: Through the telephone follow-up any reported episode which includes three or more of the following: fever, cough, sore thought, extreme fatigue, or loss of smell/loss of taste). Following an infection, the person will re-enter the at-risk-population 2 weeks later.

c) Mortality: All-cause mortality: Death due to any cause during the follow-up period. If there are cases of inconsistency between different sources of information, the HDSS information will be considered superior.

d) Hospital admission: Hospital admission for infectious disease. Self-reported admission with symptoms or diagnosis classified as caused by infection. Analysed as a repeated event. Following an admission, the person will re-enter the at-risk-population 2 weeks later.

e) Consultations for infectious disease: Consultation for infectious disease registered at one of the two health centres. Analysed as a repeated event. Following a consultation, the person will re-enter the at-risk-population 2 weeks later.

f) Reported consultations: Any consultation for infectious disease reported to have occurred at any health facility.

1.6 Statistical analyses

1.6.1 Statistical model

The outcome rates will be compared by randomisation arm in Cox proportional hazards models, with time since randomization as the underlying time, to provide hazard ratios (HRs) with 95% CIs. The main results will be adjusted for age (in 5-year age-bands) and zone (based on which the randomisation was stratified). Clustering within houses will be adjusted for. With the exception of the primary outcome, data will be analysed as a multiple failure time data set. All statistical tests will be 2-tailed and p≤0.05 considered statistically significant.

Prior to implementing the trial, stories of experimental COVID-19 vaccines planned to be tested in Africa had been circulating on social media, and many perceived the OPV, which we were providing in the present trial, as one of these experimental vaccines. We have therefore encountered high refusal rates, especially in the intervention arm. As this may have resulted in baseline imbalances, we will present both the 'crude' result (only adjusted for age and zone) and adjusted results where we will adjust for background factors which affect the estimated effect of OPV on the primary outcome by more than 10%. The background factors to be investigated are presented in Box 1.

In subsequent analyses of the trial dataset, we intend to use targeted maximum likelihood estimation (TMLE) (section 2.6.2). These analyses will be reported later.

1.6.2 Multiple testing

P-values will not be corrected for multiple testing. The secondary outcomes are testing the robustness of the findings, by examining if the pattern is similar 1) when milder illness is included, 2) with narrower COVID-19 symptom classifications, and 3) for the separate components of the composite outcome. Consequently, p<=0.05 will not be employed as a threshold for statistical significance for secondary outcomes.

1.6.3 Missing data

No outcome data will be imputed. However, for a particular event, we will assume that it was not caused by an infection if no information is obtained. Some dates of events may be imprecise (e.g., unknown date of consultation or only month of event known) – such dates will be allocated to the midpoint of the range of possible dates. As all individuals are followed for mortality through the HDSS, individuals whom we were not able to contact by telephone will still contribute to the assessment of the main outcome.

1.6.4 Test for proportional hazards

To test the proportional hazards assumption, a required assumption of the Cox regression, we will perform formal significance tests based on Schoenfeld residuals. In addition, we will assess proportionality by allowing the hazard ratio to interact with the underlying timescale to identify a possible time trend. Finally, we will assess proportionality graphically via log-log survival curves.

Significance tests based on Schoenfeld residuals will be performed via the Stata command *estat phtest, detail* leading to both a global test and a test for each covariate, the latter being relevant only when we study effect modifications. Presentation of log-log survival curves will be undertaken via *stphplot.* Finally, possible interactions between hazard ratios and the underlying time scale will be further investigated via the *stcox* procedure and the *tvc()* option. For the models including effect modifications we will construct a new interaction variable (i.e., a four-level variable representing the interaction) such that a graphical assessment of proportionality can be undertaken assessing the four-level variable in a log-log survival plot.

If we identify evidence for non-proportionality, we will still report the marginal hazard ratios but supplement this measure by hazard ratios for 2-3 properly selected categorical time-periods identified based on the aforementioned proportionality investigations.

2. Analyses

2.1 Baseline comparison

Descriptive statistics: We will describe participant flow by group allocation in a flowchart. For participants included in the main analysis, we will describe background factors. Background factors will be summarised by counts (percentages), means (standard deviation) or medians (interquartile range) as appropriate. Information on the proportion with missing information will be provided.

Box 1: Background factors which will be presented by intervention and control group

Suburb (group of zones)*
Age*
Sex
Formal education (highest completed grade)
Whether signed or fingerprinted the consent form
Visual classification of weight
Prior chronic illness (major types: Hypertension/Diabetes)
Medicine intake in the month prior to enrolment
Admissions during the past 3 months

*All effect estimates will be presented adjusted for age and zone. For the other listed variables, they will be included as co-variates if adjusting for them changes the effect estimate by at least 10%

2.2 Primary analysis of primary outcome

The rates of the primary outcome (a composite outcome of the first of death, hospitalisation for infection and/or consultation for infection at the health centre) within 6 months after enrolment will be compared in Cox proportional hazards models with time since enrolment as the underlying timescale.

The primary analysis of the primary outcome is described in more detail in table 1.

Table 1: Primary analysis of primary outcome

Population	Per-protocol population
Observation period	From: enrolment To: 183 days after enrolment Censoring: None
Failure definition	Death or first post-enrolment admission for infection and/or consultation for infection registered at a health centre.
Statistical tools	Cox-proportional hazards model, adjusted for the zone (stratification variable in the randomisation) and age. Robust standard error to adjust for same-house clustering.
Outline stata code	*stset outdate, f(combinedoutcome=1) origin(dateenrol) enter(dateenrol) exit(dateenrol+183)* *stcox group b11.zone b50.agegroup b1.box1var1 b1.box1var2 …, vce(cl house)* Where [box1var1] [box1var2] etc. are the background factors from Box1 which affect the [group]-estimate by at least 10% Check of proportional hazards assumption[1]: *estat phtest, detail* *stphplot, adj(zone agegroup box1var box2var) strata(group)*[2] stcox group, strata(*zone agegroup box1var box2var*) vce(cl house) /// tvc(group) texp(_t)

2.3 Analyses of secondary outcomes

For our secondary outcomes a) episodes with self-reported infectious disease morbidity reported through the monthly follow-up calls, b) episodes with self-reported infectious disease morbidity suspected to be caused by COVID-19 (three or more of the following: fever, cough, sore thought, extreme fatigue, loss of smell and/or taste) and f) reported consultation for infectious disease, we will analyse repeated events data sets including all individuals for whom information was obtained through the telephone follow-up.

For the separated components of the composite outcome the population depends on the outcome assessed: c) mortality is for all individuals, d) hospital admissions for all individuals for whom a telephone follow-up was conducted and e) recorded consultation for all individuals alive in the study area.

The analyses of the secondary outcomes are described in more detail in table 2, 3 and 4.

[1]If we identify evidence for non-proportionality, we will still report the marginal hazard ratios, but supplement this estimate by hazard ratios for 2-3 properly selected categorical time-periods identified based on the aforementioned proportionality investigations.

[2] If there is indication of non-proportionality based on assessment of the log-log curves, we will test for non-proportionality by replacing the term texp(_t) with texp(_t>s) for specific values of s.

Table 2: Analyses of secondary outcomes (a, b, d, f) – reported morbidity outcomes

Population	Per-protocol population
Observation period	From: enrolment To: 183 days after enrolment Censoring: Death, Date of loss to follow-up (last telephone interview)
Failure definition	a) Self-reported infectious disease morbidity. b) Self-reported infectious disease morbidity suspected to be caused by COVID-19 d) Hospital admission for infectious disease f) Reported consultation for infectious disease Repeated events. Episodes within 2 weeks of each other will be considered as related to the same infection. Thus, a trial participant will re-enter the population-at-risk 14 days after the episode start, provided that there has been a period without reported symptoms in the meantime.
Statistical tools	Repeated Cox-proportional hazards model, adjusted for the zone (stratification variable in the randomisation), age and box1 factors. Robust standard error to adjust for same-house clustering.
Outline stata code for analysis:	*stset outdate2[3], f(outcome=1) origin(dateenrol) time0(datestart) exit(censoring_date) id(study number)* *stcox group b11.zone b50.agegroup b1.box1var1 b1.box1var2 …, vce(cl house)* Where [box1var1] [box1var2] etc. are the background factors from Box1 which affect the [group]-estimate for the main outcome by more than 10%

Table 3: Analyses of secondary outcomes (c) - mortality

Population	Per-protocol population
Observation period	From: enrolment To: 183 days after enrolment Censoring: Migration
Failure definition	Death due to any cause
Statistical tools	Cox-proportional hazards model, adjusted for the zone (stratification variable in the randomisation), age and box1 factors. Robust standard error to adjust for same-house clustering.
Outline stata code for analysis:	*stset outdate, f(death=1) origin(dateenrol) enter(dateenrol) exit(dateenrol+183)* *stcox group b11.zone b50.agegroup b1.box1var1 b1.box1var2 …, vce(cl house)* Where [box1var1] [box1var2] etc. are the background factors from Box1 which affect the [group]-estimate for the main outcome by at least 10%

[3] Where outdate2 is the date of debut of symptoms and datestart is the date of enrolment or 14 days after a previous episode.

Table 4: Analyses of secondary outcomes (e): Recorded consultation due to infectious disease.

Population	Per-protocol population
Observation period	From: enrolment To: 183 days after enrolment Censoring: Death
Failure definition	Recorded consultation due to infectious disease. Repeated events. Episodes within 2 weeks of each other will be considered to be related to the same infection. Thus, a trial participant will re-enter the at-risk-population 14 days after the episode start, provided that there has been a period without reported symptoms in the meantime.
Statistical tools	Repeated Cox-proportional hazards model, adjusted for the zone (stratification variable in the randomisation), age and box1 factors. Robust standard error to adjust for same-house clustering.
Outline Stata code for analysis:	*stset outdate2[4], f(outcome=1) origin(dateenrol) time0(datestart) exit(censoring_date) id(study number)* *stcox group b11.zone b50.agegroup b1.box1var1 b1.box1var2 …, vce(cl house)* Where [box1var1] [box1var2] etc. are the background factors from Box1 which affect the [group]-estimate for the main outcome by more than 10%

[4] Where outdate2 is the date of debut of symptoms and datestart is the date of enrolment or 14 days after a previous episode.

2.4 Additional analyses of primary outcome.

2.4.1. Effect-modifier analyses of primary outcome. Background factors known at enrolment.

We will assess whether the effect of the intervention on the primary effect measure is modified by the potential effect modifiers sex, presence of a BCG and/or smallpox vaccination scar, age, visual weight-score, and season of enrolment.

Table 5. Potential effect modifiers of the primary outcome – fixed during observation period

Population	Per-protocol population
Observation period	From: enrolment To: 183 days after enrolment Censoring: None
Potential effect modifiers	Sex Presence of a BCG vaccination scar Presence of a smallpox vaccination scar Age Visual weight-score Season of enrolment (Rainy: June-November, Dry: December-May)
Failure definition	Death or first post-enrolment admission for infection and/or consultation for infection.
Statistical tools	Cox-proportional hazards model with interaction term, adjusted for the zone (stratification variable in the randomisation), age and box1 factors. Robust standard error to adjust for same-house clustering.
Outline stata code for analysis:	*stset outdate, f(combinedoutcome=1) origin(dateenrol) enter(dateenrol) exit(dateenrol+183)* *stcox group#EfM EfM b11.zone b50.agegroup b1.box1var1 b1.box1var2 …, vce(cl house)* Where EfM is the potential effect modifier (categorical variable) and [box1var1] [box1var2] etc. are the background factors from Box1 which affect the [group]-estimate for the main outcome by more than 10%

2.4.2. Effect-modifier analyses of primary outcome. Changing effects during follow up

In the main analyses, observation time will be censored at 183 days after enrolment. However, the effect of OPV may vary over the 6 months and by season.

Furthermore, the risk of COVID-19 may also vary during the 6 months of follow-up: During 2020 and 2021, Guinea-Bissau experienced three waves of COVD-19 infections, during which the risk of COVID-19 has been elevated (figure). Based on this, we have identified periods of higher transmission: Before 1 October 2020, 1 January - 1 April 2021 and after 1 July 2021.

Finally, health staff have been on strikes during a large part of 2021. We will assess whether effects differ by periods of observation.

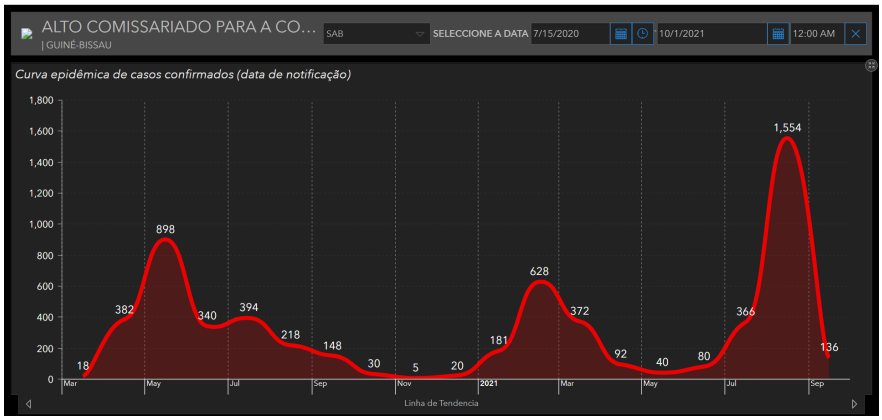

Figure: Number of confirmed COVID-19 cases per week in the Bissau sector (source www.ACCOVID.com).

Time varying effects will be investigated in the models described below.

Table 6. Potential effect modifiers of the primary outcome – changing effects during observation period

Population	Per-protocol population
Observation period	From: enrolment To: 183 days after enrolment Censoring: None
Potential effect modifiers	a) First 3 vs subsequent 3 months of follow up b) Season of follow-up (Rainy: June-November, Dry: December-May) c) Periods of intense transmission (Figure) d) Periods with health staff on strike
Failure definition	Death or first post-enrolment admission for infection or consultation for infection.
Statistical tools	Cox-proportional hazards model with interaction term, adjusted for the zone (stratification variable in the randomisation), age and box1 factors. Robust standard error to adjust for same-house clustering.
Outline stata code For analysis:	*stset outdate, f(combinedoutcome=1) origin(dateenrol) enter(dateenrol) exit(dateenrol+183) id(studynumber)* a) Time since enrolment: *g splitdate1=dateenrol+91 if dateenrol+_t >splitdate1* *stsplit time, at(0) after(splitdate1)* *replace time=time+1* *recode time .=0* *stcox group#time time b11.zone b50.agegroup b1.box1var1 b1.box1var2 …, vce(cl house)* Where EfM is the potential effect modifier (categorical variable) and [box1var1] [box1var2] etc. are the background factors from Box1 which affect the [group]-estimate for the main outcome by more than 10% b) Splitting the observation time 1 December 2020 and 1 June 2021 to define rainy season (before 1 December and after 1 June) and dry season

	(December-May) This variable "rain" (0/1) will be included as an effect modifier. *stcox group#rain rain b11.zone b50.agegroup b1.box1var1 b1.box1var2 …, vce(cl house)* Where EfM is the potential effect modifier (categorical variable) and [box1var1] [box1var2] etc. are the background factors from Box1 which affect the [group]-estimate for the main outcome by more than 10% c) Splitting the observation time at 1 October 2020, 1 January, 1 April 2021 and 1 July 2021 to define exposed time intervals: Before 1 October 2020, 1 January-1 April 2021 and after 1 July 2021. This variable "expos" (0/1) will be included as an effect modifier. *stcox group#expos expos b11.zone b50.agegroup b1.box1var1 b1.box1var2 …, vce(cl house)* Where EfM is the potential effect modifier (categorical variable) and [box1var1] [box1var2] etc. are the background factors from Box1 which affect the [group]-estimate for the main outcome by more than 10% d) Splitting the observation time into periods affected and not affected by strikes among health staff. *stcox group#strike stike b11.zone b50.agegroup b1.box1var1 b1.box1var2 …, vce(cl house)* Where EfM is the potential effect modifier (categorical variable) and [box1var1] [box1var2] etc. are the background factors from Box1 which affect the [group]-estimate for the main outcome by more than 10%

2.5 Additional analyses of secondary outcomes.

2.5.1. Effect-modifier analyses of secondary outcomes

Analogous to effect modifications analyses for the primary outcomes (Tables 5 and 6), we will assess whether the same potential effect modifiers are effect modifiers of the secondary outcomes (Tables 2-4).

2.6. Further sensitivity analyses

2.6.1 Narrower outcome definitions

In sensitivity analyses we will explore if the conclusions are robust to:

Narrower definitions of the planned outcomes:

- Suspected infectious deaths rather than all cause deaths
- Severe infections with reported loss of weight
- Infections with reported COVID-19 diagnoses, tests performed and positive tests

2.6.2 Alternative analytical approaches

Due to the potential imbalance which may have resulted due to the differential refusal rates, we will in subsequent analyses use targeted maximum likelihood estimation[2,3] (TMLE) in a re-analysis of primary and secondary outcomes. The modelling step will include the categorical variables sex, visual weight-classification (1-10), chronic illness, symptoms on the day of enrolment, medicine intake in the past month, ethnic group, whether signed or fingerprinted the consent form, and the continuous variable, age.

Specification of the models used will be guided by a machine learning algorithm (Super Learner)[4] using both a default library of prediction models as well as a more elaborate library.

3. References

1. Cohen E, Bernard JY, Ponty A, et al. Development and Validation of the Body Size Scale for Assessing Body Weight Perception in African Populations. *PloS one* 2015; **10**(11): e0138983.
2. van der Laan MJ, Rubin D. Targeted Maximum Likelihood Learning. *The International Journal of Biostatistics* 2006; **2**(1).
3. van der Laan MJ, Rose S. Targeted Learning: Causal Inference for Observational and Experimental Data Berlin: Springer.
4. Polley EC, van der Laan MJ. Super Learner In Prediction. U.C. Berkeley Division of Biostatistics: Working Paper 266., 2010.