CLINICAL STUDY REPORT SYNOPSIS

An individually randomized, double-blind, placebo-controlled phase 2b trial (OEV-128) examining the efficacy of oral inactivated ETVAX vaccine to prevent enterotoxigenic *E. coli*-associated diarrhoea in Gambian children ages 6 to 18 months.
IRB Ref: 17868

Investigational Product:	**ETVAX**
Indication:	**Enterotoxigenic *Escherichia coli* (*E. coli*)-associated diarrhoea**
Development Phase:	**Phase 2b**
Study Initiation Date:	**22nd February 2021**
Study Completion Date:	**31st October 2023**
Report Completion Date:	**20th June 2024**
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Responsible for Sponsor:	Björn Sjöstrand, Chief Executive Officer (CEO) Email: bjorn.sjostrand@scandinavianbiopharma.se

GCP STATEMENT
This study was conducted in compliance with Good Clinical Practices, according to the ICH Harmonised Tripartite Guideline.

CONFIDENTIALITY STATEMENT
This clinical study report is confidential and the property of Sponsor and may not be used, disclosed or published without their consent.

2 SYNOPSIS

Title of Study:
An individually-randomized, double-blind, placebo-controlled phase 2b trial (OEV-128) examining the efficacy of oral inactivated ETVAX vaccine to prevent *enterotoxigenic E. coli*-associated diarrhoea in Gambian children ages 6 to 18 months.

Co-ordinating Investigator:
Dr. Jahangir Hossain

Study Centre(s):
The study subjects were recruited from the catchment area of Farafenni Hospital, a tertiary hospital in the central part of The Gambia. There were 4 vaccination centres and 10 surveillance centres:

Farafenni Hospital/Farafenni RCH Clinic (vaccination and surveillance)
Ngayen Sanjal HC (vaccination and surveillance)
Njaba Kunda HC / Nookunda HC (vaccination and surveillance)
Kerewan HC (vaccination and surveillance)
Sara Kunda HC (surveillance)
Iliassa HC (surveillance)
Salikene HC (surveillance)
Kuntair HC (surveillance)
Soma HC (surveillance)
Kaur HC (surveillance)

Publication (reference):
N/A

Studied Period (years):	**Clinical Phase:**
(date of first enrolment) 22nd February 2021 (date of last completed) 31st October 2023	Phase 2b

Objectives:
Primary

- Safety: To evaluate the safety and reactogenicity of three oral doses of ETVAX administered to children ages 6 to 18 months on days 1, 15, and 90.

- Efficacy: To measure the protective efficacy of three vaccine doses of ETVAX given on days 1, 15 and 90 to children ages 6 to 18 months, from 7 days after receiving the third dose of vaccine or placebo and followed for an average of 18 months for the first clinically significant (moderate-to-severe) ETEC diarrhoeal episodes associated with *E.coli* heat-labile enterotoxin (LT)- or LT/ *E.coli* heat-stable (ST)-enterotoxin, or ST with at least one colonization factor (CF) (CFA/I, CS3, CS5, CS6).

Secondary

- Efficacy: To further measure the protective efficacy of ETVAX by evaluating two doses, diarrhoeal aetiology, severity, presence of co-pathogens / co-infections and acute diarrhoeal episode with three or more loose stools in 24 hours.

- Immunogenicity: To assess vaccine induced serum IgA antibody responses against LTB, CFA/I, CS3, CS5, and CS6 and IgG responses against LTB in a subset of Gambian children ages 6 to 18 months.*

* This objective was rewritten for further clarification:
To compare the serum antibody IgA responses against vaccine antigens (CFA/1, CS3, CS5, CS6 and LTB) and IgG responses against LTB between the vaccine and the placebo groups, and between the subjects enrolled in the beginning of the study and the end of the study within the vaccine group.

Exploratory

- To explore the impact on the protective efficacy of acute diarrhoeal episodes following three doses of ETVAX in presence/ absence of the most frequently detected co-pathogens / co-infections.
- To explore the impact on the protective efficacy of acute diarrhoeal episodes following at least two doses of ETVAX.
- To explore the impact on the protective efficacy of acute diarrhoeal episodes following three doses of ETVAX, expressing any ETEC CFs and ETEC toxins.

Methodology:

This was a randomized, double-blind, placebo-controlled paediatric phase 2b study testing the efficacy of ETVAX vaccine, a multi-valent, oral whole-cell inactivated *E. coli* vaccine overexpressing *enterotoxigenic E-coli* (ETEC) colonisation factors (CFA/I, CS3, CS5 and CS6) and a hybrid protein of the B-subunits of the heat-labile *E.-coli* and cholera toxins (LCTBA), with a double-mutant heat-labile enterotoxin adjuvant (dmLT). One adult dose of ETVAX contained 10^{11} bacteria, 1mg LCTBA and 10µg dmLT. In the study each subject received three oral ETVAX vaccine 1/4 doses or placebo on study days 1, 15, and 90.

A total of 4936 healthy children ages 6-18 months, 2468 each in the vaccine or placebo group, were randomized. Informed consent was obtained at enrolment and Inclusion and exclusion criteria were assessed. At the clinic, eligible subjects were randomized to receive vaccine or placebo with 1:1 allocation. The parents were requested to bring their child for a second dose on study day 15, and for a third dose on study day 90.

Following each vaccination, subjects remained at the clinical study site for at least 30 minutes for post-vaccination observation. Clinical study staff followed each of the 1st 350 enrolees during home visits to assess reactogenicity including both solicited and unsolicited adverse events during the first week after each vaccine dose. Serious Adverse Events (SAEs) were monitored during the entire study period. Recording, management and reporting of adverse events (AEs) and SAEs was done according to the MRCG unit's standard operating procedure and were reported to the sponsor, Data Safety and Monitoring Board (DSMB) and local ethics committee.

Systemic immune responses were measured in a subset of subjects (n=150); 75 subjects among the first 750 enrolees were selected randomly at the beginning of enrolment, e.g., on average every 10th child until 75 children were selected. To show the consistency of vaccine responses over the length of the vaccination period, another 75 subjects were selected in the same way among the last two cohorts and similarly tested for immune responses against the vaccine antigens. Health personnel at the study site obtained blood on study day 1 (pre-dose) and 7 days after the second and third dose.

Number of Subjects (total and for each dosage):

5253 subjects were screened.
4936 subjects were randomised (2468 subjects randomised to ETVAX vaccine treatment and 2468 subjects randomised to placebo (Sodium Bicarbonate buffer) treatment.

Diagnosis and Criteria for Inclusion:

Inclusion criteria

1. Healthy children 6 to 18 months old at the time of enrolment.
2. Generally good health over the 7 days before enrolment and the day of 1st dose of vaccine / placebo.
3. Parent properly informed about the study, able to understand it and sign or thumb print the informed consent form.
4. Parent and child available for the entire study period of the study and reachable by study staff throughout the entire follow-up period.

Exclusion criteria

1. Presence of any significant known systemic disorder (cardiovascular, pulmonary, hepatic, renal, gastrointestinal, endocrine, immunological, dermatological, neurological, cancer or autoimmune disease) as determined by medical history and / or physical examination which would endanger the subject's health or is likely to result in non-conformance to the protocol.

2. History of congenital abdominal disorders, intussusception, abdominal surgery or any other congenital disorder or presence of a significant medical condition that in the opinion of the Investigator precludes participation in the study. Known or suspected impairment of immunological function based on medical history and physical examination.
3. Clinical evidence of active gastrointestinal illness and acute disease at the time of enrolment.
4. Participation in research involving another investigational product (defined as receipt of investigational product) 30 days before planned date of first vaccination.
5. History of receiving any other vaccines given within 7 days before ETVAX vaccination.
6. Antibiotics administered within 7 days before ETVAX vaccination.
7. History of febrile illness within 48 hours prior to vaccination or documented fever at the time of immunisation (fever is defined as a temperature ≥ 37.5°C) on infrared thermometer measurement.
8. Prior receipt of Dukoral®, an oral cholera vaccine, or any ETEC vaccine candidate.
9. Prior receipt of a blood transfusion or blood products, including immunoglobulins.
10. Current use of iron or zinc supplements within the past 7 days.
11. Current use of antacids (H2 blockers, omeprazole, over the counter (OTC) agents) or immunosuppressive drug within the past 7 days.
12. Any condition that in the opinion of the PI might jeopardize the safety of study subjects or interfere with the evaluation of the study objectives.
13. History of diarrhoea during 7 days before vaccination.
14. Acute disease at the time of enrolment or during the 3 days prior to enrolment.
15. Subject's parents not able, available or willing to accept active follow-up by the study staff.
16. History of chronic administration (defined as more than 14 days) of immunosuppressant medications, including corticosteroids.
17. Medically significant malnutrition, defined as moderate or more severe malnutrition (weight for height z-score <-2.0).

Investigational Product, Dose, Mode of Administration, Batch No.:
In the study the subjects were administered 1/4 of an adult dose of ETVAX vaccine and received three oral vaccine doses on study days 1, 15, and 90. The ETVAX vaccine consists of four different inactivated *E. coli* strains expressing the most prevalent ETEC CFs, i.e. CFA/I, CS3, CS5 and CS6, a hybrid protein LCTBA and the adjuvant double mutant LT (dmLT). Throughout the report the ETVAX vaccine is referred to as "vaccine".

Vaccine: 1 batch used (FBH1601E)
dmLT: 1 batch used (01/15 DP)
Phosphate Buffer Solution (PBS): 1 batch used (8243062)
Buffer sachets: 2 batches (SH816A and SH909A)

Duration of Treatment:
There were 3 months of vaccinations followed by passive surveillance for at least 12 months.

Reference Therapy, Dose, Mode of Administration, Batch No.:
Placebo (Sodium Bicarbonate Buffer) on study days 1, 15 and 90.
Buffer sachets: 2 batches (SH816A and SH909A).

Criteria for Evaluation:
Primary Efficacy Endpoint:

- The first episode of clinically significant moderate-to-severe acute diarrhoea with four or more loose or liquid stools associated with culture-detected ETEC expressing LT- or LTST-enterotoxin, or ST with at least one CF (CFA/I, CS3, CS5, CS6) without co-pathogens** among children 6 to 18 months of age receiving two doses and a booster dose (three doses) and detected from 7 days after receiving the third dose of vaccine or placebo, on average 18 months of follow-up.

** In this study *diarrhoea without co-pathogens / co-infections was defined as isolation of ETEC without presence of culture-confirmed Shigella spp (Shigella), PCR-confirmed rotavirus, norovirus GII, and Cryptosporidium spp (Cryptosporidium)*

Secondary Efficacy Endpoints:

1. The first episode of clinically significant moderate to severe acute diarrhoea, with four or more loose or liquid stools associated with culture-detected ETEC expressing LT- or LTST-enterotoxin, or ST with at least one CF (CFA/I, CS3, CS5, CS6) without co-pathogens** among children receiving two doses of ETVAX or placebo detected from 7 days after receiving the second dose to the date of the third dose or in case no third dose is given through the follow-up period.

2. The first episode of clinically significant (moderate to severe) acute diarrhoea with four or more loose or liquid stools associated with culture-detected ETEC regardless of CFs or enterotoxins without co-pathogens** among children 6 to 18 month of age receiving three doses of vaccine or placebo detected from 7 days after receiving the third dose through, on average, 18 months of follow-up.

3. The first episode of clinically significant (moderate to severe) acute diarrhoea with four or more loose or liquids stools associated with culture-detected ETEC regardless of CF or enterotoxin and regardless of co-pathogens / co-infections among children 6 to 18 months of age receiving three doses of vaccine or placebo detected from 7 days after receiving the third dose through, on average, 18 months of follow-up.

4. The first episode of clinically significant (moderate to severe) acute diarrhoea with four or more loose or liquids stools associated with culture-detected ETEC expressing LT- or LTST-enterotoxin, or ST with at least one CF (CFA/I, CS3, CS5, CS6) regardless of co-pathogens / co-infections among children 6 to 18 months of age receiving three doses of vaccine or placebo detected from 7 days after receiving the third dose through, on average, 18 months of follow-up.

5. The first episode of clinically significant (moderate to severe) acute diarrhoea with four or more loose or liquids stools regardless of aetiology among children 6 to 18 months of age receiving three doses of vaccine or placebo detected from 7 days after receiving the third dose through, on average, 18 months of follow-up.

6. The first episode of clinically significant (severe**)** acute diarrhoea with four or more loose or liquids stools associated with culture-detected ETEC expressing LT- or LTST-enterotoxin, or ST with at least one CF (CFA/I, CS3, CS5, CS6) without co-pathogens** among children receiving three doses of vaccine or placebo detected from 7 days after receiving the third dose through, on average, 18 months of follow-up period.

7. The first episode of acute diarrhoea with three or more loose or liquid stools and with signs/ symptoms of dehydration associated with ETEC expressing LT- or LTST-enterotoxin, or ST with at least one CF (CFA/I, CS3, CS5, CS6) without co-pathogens** among children receiving three doses of vaccine of placebo detected from 7 days after receiving the third dose through, on average, 18 months of follow-up period.

8. The first episode of acute diarrhoea with three or more loose or liquid stools and with signs/ symptoms of dehydration associated with ETEC expressing LT- or LTST-enterotoxin, or ST with at least one CF (CFA/I, CS3, CS5, CS6) without co-pathogens** from 7 days after the second dose up to the date of third dose or in case no third dose is given through the follow-up period.

** In this study *diarrhoea without co-pathogens / co-infections was defined as isolation of ETEC without presence of culture-confirmed Shigella, PCR-confirmed rotavirus, norovirus GII, and Cryptosporidium*

Exploratory Efficacy Endpoints:

1. The first episode of clinically significant (moderate to severe) acute diarrhoea with four or more loose or liquids stools) associated with culture-detected ETEC expressing LT- or LTST-enterotoxin, or ST with at least one CF (CFA/I, CS3, CS5, CS6) with absence or presence of the most frequently detected co-pathogens / co-infections. Further analyses were performed in the same manner where groups (bacteria, viruses, parasites) of co-pathogens / co-infections were allowed / not allowed.

2. The first episode of clinically significant (moderate-to-severe) acute diarrhoea with four or more loose or liquid stools associated with culture-detected ETEC expressing LT- or LTST-enterotoxin, or ST with at least one CF (CFA/I, CS3, CS5, CS6) without co-pathogens** (defined as isolation of ETEC without presence of culture confirmed Shigella, PCR-confirmed rotavirus, norovirus GII or *Cryptosporidium*) among children 6 to 18 months of age receiving at least two doses and detected from 7 days after receiving the last dose of vaccine or placebo through, on average, 18 months of follow-up.

3. The first episode of acute diarrhoea with three or more loose or liquid stools and with signs / symptoms of dehydration associated with culture-detected ETEC expressing LT- or LTST-enterotoxin, or ST with at least one CF (CFA/I, CS3, CS5, CS6) without co-pathogens** among children 6 to 18 months of age receiving at least two doses of ETVAX and detected from 7 days after receiving the last dose of vaccine or placebo through, on average, 18 months of follow-up.

4. The first episode of clinically significant (moderate-to-severe) acute diarrhoea with four or more loose or liquids stools associated with culture-detected ETEC expressing LT- or LTST-enterotoxin, or ST with at least one CF (CFA/I, CS3, CS5, CS6, or vaccine cross-reactive CFs CS7, CS14, CS17) without co pathogens** among children 6 to 18 months of age receiving two doses and a booster dose (three doses) and detected from 7 days after receiving the third dose of vaccine or placebo through, on average, 18 months of follow-up.

** In this study *diarrhoea without co-pathogens/ co-infections was defined as isolation of ETEC without presence of culture-confirmed Shigella, PCR-confirmed rotavirus, norovirus GII, and Cryptosporidium*

Post Hoc Efficacy Endpoint (combination of secondary endpoint 4 and 7):

- The first episode of acute diarrhoea with three or more loose or liquid stools and signs/symptoms of dehydration associated with culture-detected ETEC expressing LT- or LTST-enterotoxin, or ST with at least one CF (CFA/I, CS3, CS5, CS6) regardless of co-pathogens / co-infections among children receiving three doses of vaccine or placebo detected from 7 days after receiving the third dose through, on average, 18 months of follow-up.

Primary Safety Endpoint:

- Occurrence of serious adverse events (SAEs) among all enrolled children from receipt of the first vaccine dose to end of study and among the reactogenicity cohort (n=350), reactogenicity and adverse events (AEs) identified during 7 days after receiving each dose of vaccine.

Secondary immunogenicity Endpoints: ***

- In the immunogenicity subset of subjects (n=150), the proportion of individuals mounting a serum IgA antibody response detected by ELISA to the different vaccine CFs and LTB as well as responses to at least one, two, three, four and five of the five primary vaccine antigens (≥ two-fold increase in antibody titres between baseline and after the second and/or third vaccine dose).

- The proportion of subjects (n=150), in the immunogenicity subset, mounting a serum IgG antibody response detected by ELISA against LTB (≥ two-fold increase in antibody titres between baseline and after the second and/or third vaccine dose.

*** The secondary immunology endpoints were rewritten for further clarification:

- Antibody titres at baseline and after the second and / or third vaccine dose.
- Fold rise in antibody titres between baseline and after the second and/or third vaccine dose.
- Two -fold increases in antibody titres between baseline and after the second and / or third vaccine dose.
- Responses (≥ two-fold increase) to at least one, two, three, four and five of the five primary vaccine antigens

Statistical Methods:

Sample Size

The sample size calculation was based on the primary analysis, per protocol of efficacy against clinically significant (moderate-to-severe) ETEC diarrhoea occurring 7 days from the third dose and, on average,

during the 18 months study period among children receiving two doses 14 days apart and a third dose at 90 days (3 months) after the first dose. For sample size, the incidence of clinically significant (moderate-to-severe) vaccine preventable ETEC diarrhoea among unvaccinated children was assumed to be 1.87% over 18 months of follow-up. Assuming 60% efficacy (incidence of 0.75% in the intervention arm), a 1-sided p-value at 0.05 based on a chi-square statistic with 80% power, 85% Investigational Product (IP) coverage, expected dropout of 12%, and 30% would not seek health care for clinically significant (moderate-to-severe) diarrhoea, the number of persons per group was 2,468 children or 4936 children in total. A supplemental sample size calculation was performed for the Cox score test in proportional hazards regression, assuming a 1.31% event probability, a test hazard ratio of 0.4 and a test standard deviation of 0.5 (consistent with a 1:1 randomization). With a total sample size of 4936 children, assuming 85% IP coverage and 30% not seeking care for clinically significant diarrhoea, the size of the analysis population was expected to be 2937 subjects. Using a two-sided test and a significance level of 0.05 the power was 81%, consistent with the initial sample size calculation.

In this study, 350 volunteer children aged 6 to 18 months were under active surveillance for reactogenicity / safety and given an allocation ratio of 1:1 (vaccine: placebo) to have 175 children who received ETVAX. Based on the probability of a single event using the binomial distribution $[(1-p)^n]$, we had 80% chance to detect a vaccine related adverse event that occurred once in 100 studies. Following these guidelines, and with 350 children, there should be sufficient power to detect statistically significant differences of mild adverse events between the two treatment arms (only mild AEs were detected in previous safety studies). For AEs occurring at a prevalence of 2% in the placebo arm (such as mild vomiting), there was at least 90% power to detect a statistically significant difference if the prevalence in the vaccine arm was 10%.

Populations

Enrolled population

The enrolled population consists of screened participants who provided informed consent, regardless of the subject's randomization and treatment status in the study.

Safety analysis population

The safety analysis population consists of subject's who received at least one dose of the vaccine or placebo regardless of treatment received, noncompliance, protocol deviations, and withdrawal. Subjects were allocated to the actual treatment received in case of incorrect dosing. If a subject by mistake received both vaccine and placebo, the subject was allocated to the vaccine group. The Safety Analysis population was used to evaluate AEs, (laboratory safety data), and treatment compliance/administration.

Intention to treat population

The intention to treat (ITT) population consists of subjects who received at least one dose of the vaccine or placebo regardless of treatment received, noncompliance, protocol deviations, and withdrawal. Subjects were allocated to the randomized treatment in case of incorrect dosing.

Modified intention to treat population

For primary, secondary and exploratory efficacy endpoints, the modified intention to treat (mITT) population consists of subjects who received at least the doses of the vaccine or placebo indicated in the definition of the endpoint, regardless of treatment received, noncompliance, protocol deviations, and withdrawal. Subjects were allocated to the randomized treatment in case of incorrect dosing.

Per protocol population

The primary analysis was performed in the per protocol (PP) population. The PP population consists of subjects who were randomized and correctly received 3 doses (two doses and a booster dose) of investigational product (IP). Subjects with major protocol deviations considered to affect the efficacy endpoints were excluded from the PP population. Decisions on exclusion from the PP population were made prior to database lock. For endpoints assessed before the third dose, the per protocol population consists of subjects who correctly received the first two doses of randomized treatment without major protocol deviations.

Immunological analysis population

The population for immunogenicity analysis consists of all randomized subjects who received at least

two doses of the vaccine or placebo and with a pre-immunisation blood sample and at least one post-immunisation blood sample drawn for immunogenicity.

Statistical considerations
Categorical variables were summarised with counts and percentages while continuous variables were summarised with mean, standard deviation (SD), minimum, median and maximum values. In the statistical analyses, p values less than 0.05 were considered statistically significant. No adjustment for multiplicity was performed. All tests and confidence intervals (CI) were two-sided unless stated otherwise.

Efficacy Analysis
The primary efficacy and secondary analyses were based on the PP Analysis Set. Additional supportive analysis based on the ITT Analysis Set were performed for the primary and secondary efficacy variables.

The Exploratory analyses were based on the ITT Analysis Set. Additional analysis based on the PP Analysis set were performed post hoc for some of the exploratory analyses.

The incidence of vaccine preventable outcomes (VPO) was assessed in the vaccine and the placebo groups. A survival analysis was employed to calculate the vaccine efficacy with measurement of time to first ETEC episode, subjects who were withdrawn from study were considered right censored To obtain adjusted hazard ratios of ETEC, Cox proportional hazard regression models were used after ensuring that the proportionality assumption was fulfilled for all independent variables. The percentage of vaccine protective efficacy was expressed as (1-hazard ratio) x100 along with two-sided 95% CI and p-value. Crude vaccine efficacy was calculated using methodology for relative risk.

Safety Analysis
Adverse events were identified and tabulated among all subjects (cohorts 1-6) who received at least one dose of the vaccine or placebo following the first dose until the end of the study.

All subjects (cohorts 1-6) were followed for reactogenicity including both solicited adverse events (clinically significant vital signs, acute systematic allergic reactions, and vomiting) and unsolicited adverse events up to 30 minutes after each dose.

In the first 350 randomized subjects (reactogenicity cohort / cohort 1), subjects were visited at home on days 2, 3, and 7+2 days for reactogenicity including both solicited adverse events (diarrhoea, pyrexia, urticaria, vomiting, or other reactogenicity events, i.e. events occurring within 7 days and scored as related to reactogenicity) and unsolicited adverse events.

All subjects (cohorts 1-6) were followed for unsolicited adverse events until the end of the study.

The proportions of subjects with adverse events, with possible treatment adverse events and serious adverse events overall, by grade / severity, relationship to IP and timing were compared between the vaccine and placebo groups. Pre-treatment and treatment emergent adverse events were also summarized. All safety analyses were descriptive; no statistical inference procedures were applied.

Immunological Analysis
The serum antibody IgA responses against vaccine antigens (CFA/1, CS3, CS5, CS6 and LTB) and IgG responses against LTB were compared per time point between the vaccine and the placebo groups, and between the subjects enrolled in the beginning of the study (enrolled June 2021 or earlier) and the end of the study (enrolled February 2022 or later) within the vaccine group. Both the magnitudes of responses (fold rises) for each antigen defined as the post immunisation antibody titres (after the 2nd and 3rd doses) divided by pre-immunisation titres (baseline) and the proportion of subjects with fold rises ≥ 2 and ≥ 4 were calculated. Geometric mean titres were summarised per response, treatment and time point. For the fold rises, the proportion of participants with fold rises ≥ 2 and ≥ 4, and the proportions of participants with IgA response to at least 1, 2, 3, 4 and 5 antigens were presented.

SUMMARY – CONCLUSIONS
Efficacy Results:

The efficacy of ETVAX for ETEC vaccine-preventable outcomes, excluding outcomes co-infected with Shigella, rotavirus, norovirus GII, or Cryptosporidium, was 26.6% (CI: -58.3 to 66.0, p=0.43), which did not meet the prespecified primary endpoint. However, when the ETEC co-infections were not excluded, efficacy increased to 48.2% (95% CI: -0.9% to 73.4%, p=0.053), approaching statistical significance. This result suggests that excluding those ETEC co-infections underestimated ETVAX efficacy and indicates that ETVAX has broader protection than anticipated.

A protocol amendment during the trial redefined diarrhoea cases as three or more loose stools rather than four loose stools in 24 hours. When this definition was used, the vaccine efficacy increased relative to the primary endpoint to 38.8% (95% CI: -28.7% to 70.9%. In a post hoc analysis this new definition was used with ETEC co-infections excluded and vaccine efficacy against ETEC episodes reached 50.9% (95% CI: 9.4-73.4, p=0.02), achieving statistically significance. Future trials will benefit from the revised definition.

While ETVAX offers 50.9% protection against ETEC cases regardless of co-infections, exploratory analyses indicated that other infections might still dilute the estimated protection afforded by ETVAX against ETEC outcomes. ETEC cases co-infected with Giardia were highly prevalent in this population. Excluding ETEC outcome co-infected with Giardia improved vaccine efficacy to 78.2% (95% CI: 36 to 92.6, p=0.006). Additionally, excluding ETEC co-infected with any parasites (Giardia and Cryptosporidium) increased efficacy to 80.6% (95% CI: 33.4 to 94.3, p=0.009). These results indicate that while ETVAX has significant broad protection, the greatest protection is afforded against ETEC uninfected with enteric parasites.

Safety Results:
The safety population included 2,474 and 2,462 subjects in the vaccine and placebo groups, respectively. Treatment-emergent adverse events were reported by 2,187 (44.3%) subjects, equally distributed between the vaccine group (1,093 subjects, 44.2%) and the placebo group (1,094 subjects, 44.4%).

The most common treatment-emergent adverse events (by body system) over the length of the study were gastrointestinal disorders (805, 16.3%) and there was no difference between vaccine (410, 16.6%) and placebo groups (395, 16.0%). The most common gastrointestinal disorder was diarrhoea (669, 13.6%) which did not differ by vaccine (335, 13.5%) and placebo subjects (334, 13.6%).

Most of the treatment-emergent adverse events were of mild to moderate severity (grade 1 or 2) and were equally distributed between the vaccine (2,195) and placebo groups (2,242). There were 57 adverse events classified as severe (grade 3) or potentially life-threatening (grade 4) and were also equally divided between treatment groups (27 vs 30). Finally, there were 13 adverse events that were fatal (grade 5), with six and seven among the vaccine and placebo groups, respectively.

Of all the adverse events, the vast majority were unrelated to treatment. There were 353 adverse events recorded as possibly related to the treatment, with 188 and 165 in the vaccine and placebo groups, respectively. The number of adverse events probably related to treatment was 31 and 29, and definitely related was 11 and 7, respectively.

There were 56 (1.1%) subjects with treatment-emergent serious adverse events (SAEs), with fewer in the ETVAX treatment group. This included 24 (1%) subjects in the vaccine group and 32 (1.3%) subjects in the placebo group. All SAEs were unrelated to the treatment.

The frequency and severity of solicited and unsolicited adverse events in the 30 minutes following dosing were mostly similar between treatment groups. However, there was a slightly increased number of subjects experiencing vomiting in the vaccine-treated group (n=44, 1.8%) compared to the placebo-treated group (n=29, 1.2%). The slight increase was not considered clinically significant.

In the reactogenicity cohort / cohort 1 (n=350) the frequency and severity of solicited and unsolicited adverse events identified during 7 days after receiving each dose of vaccine were mostly similar between the vaccine and placebo treatment groups.

Immunogenicity results
The vaccine induced comparable immune responses among Gambian children enrolled at the start and end of the study.

Three vaccine doses were more efficient than two doses in inducing significant immune responses against CFA/I, CS3, and CS5, whereas two doses were sufficient to induce strong anti-LTB immune responses.

The geometric mean fold rise for IgA and IgG against LTB were comparable, both were statistically significantly higher ($p<0.0001$) for two and for three doses of vaccine compared to placebo.

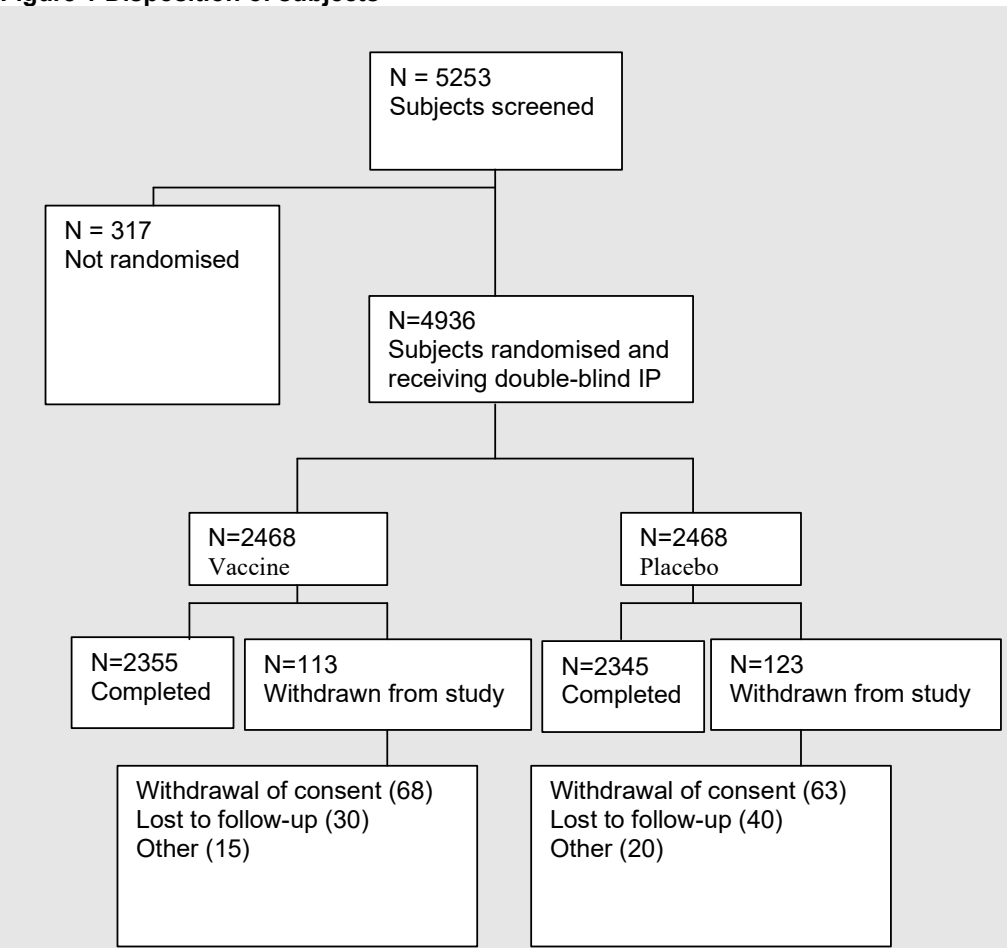
Conclusion:
The study results shows that ETVAX is safe, immunogenic, and offers protection against moderate-to-severe ETEC diarrhoea. The findings support a three-dose schedule and suggest that the vaccine may offer persisting, broader protection beyond solely ETEC diarrhoea. An ETEC vaccine has been a long-standing target by the World Health Organization (WHO). ETVAX has the potential to become a valuable public health tool for use in children in low and /or middle-income countries.

3 STUDY SUBJECTS

3.1 Disposition of Subjects

A total of 5253 subjects were enrolled in the study. Of these, 2468 subjects were randomized and treated with vaccine and 2468 patients were treated with placebo. Figure 1 shows the disposition of subjects.

Figure 1 Disposition of subjects



The first subject was enrolled on 22 FEB 2021, the last subject was enrolled on 24 JUN 2022 and the last subject completed the study on 31 OCT 2023, after which the database was locked on 19 DEC 2023 and the code was broken.

There was a total of 575 (11.6%) withdrawals from treatment (ITT population) with a similar frequency of withdrawals from the vaccine and placebo treatment groups (Table 1).

The most withdrawals were seen after the second dose, with a total of 271 (5.5%) withdrawals from treatment (137 subjects withdrew from the vaccine treatment group and 134 subjects withdrew from the placebo treatment group) and the most frequently recorded reasons for withdrawal were other (n=348, 7.0%) followed by withdrawal of informed consent (n=168, 3.4%).

Table 1 Withdrawals from treatment with respect to time and reason. ITT population

Last dosing visit attended	*Reason for missing dose of IP administration*	*Vaccine (N = 2468) [n (%)]*	*Placebo (N = 2468) [n (%)]*	*Total (N = 4936) [n (%)]*
1st Dose (Day 1)	Toxicity/Adverse Event	1 (0.04%)		1 (0.02%)
	Withdrawal of Informed Consent	40 (1.62%)	46 (1.86%)	86 (1.74%)
	Lost to follow-up	3 (0.12%)	10 (0.41%)	13 (0.26%)
	Other	12 (0.49%)	16 (0.65%)	28 (0.57%)
	Total	56 (2.27%)	72 (2.92%)	128 (2.59%)
2nd Dose (Day 15)	Toxicity/Adverse Event	0	3 (0.12%)	3 (0.06%)
	Withdrawal of Informed Consent	43 (1.74%)	38 (1.54%)	81 (1.64%)
	Lost to follow-up	14 (0.57%)	9 (0.36%)	23 (0.47%)
	Death	0	4 (0.16%)	4 (0.08%)
	Other	80 (3.24%)	80 (3.24%)	160 (3.24%)
	Total	137 (5.55%)	134 (5.43%)	271 (5.49%)
3rd Dose (Day 90)	Toxicity/Adverse Event	7 (0.28%)	8 (0.32%)	15 (0.30%)
	Withdrawal of Informed Consent	0	1 (0.04%)	1 (0.02%)
	Other	84 (3.40%)	76 (3.08%)	160 (3.24%)
	Total	91 (3.69%)	85 (3.44%)	176 (3.57%)
Total	Toxicity/Adverse Event	8 (0.32%)	11 (0.45%)	19 (0.38%)
	Withdrawal of Informed Consent	83 (3.36%)	85 (3.44%)	168 (3.40%)
	Lost to follow-up	17 (0.69%)	19 (0.77%)	36 (0.73%)
	Death	0	4 (0.16%)	4 (0.08%)
	Other	176 (7.13%)	172 (6.97%)	348 (7.05%)
	Total	284 (11.51%)	291 (11.79%)	575 (11.65%)

There was a total of 236 (4.8%) withdrawals from the study (ITT population) with a similar number of withdrawals from the vaccine and placebo treatment groups (Table 2). The most withdrawals were seen after the first dose, with a total of 83 (1.7%) withdrawals from the study and the most frequently recorded reason for withdrawal was withdrawal of consent by parent for any reason (n=70, 1.4%).

Table 2 Withdrawals from study with respect to time and reason. ITT population

Last study visit	*Primary reason for withdrawal from study*	*Vaccine (N = 2468) [n (%)]*	*Placebo (N = 2468) [n (%)]*	*Total (N = 4936) [n (%)]*
1st Dose (Day 1)	Withdrawal of consent by Parent for any reason	31 (1.26%)	39 (1.58%)	70 (1.42%)
	Lost to follow-up	3 (0.12%)	9 (0.36%)	12 (0.24%)
	Other	0	1 (0.04%)	1 (0.02%)
	Total	34 (1.38%)	49 (1.99%)	83 (1.68%)
2nd Dose (Day 15)	Withdrawal of consent by Parent for any reason	22 (0.89%)	14 (0.57%)	36 (0.73%)
	Lost to follow-up	11 (0.45%)	11 (0.45%)	22 (0.45%)
	Other	0	4 (0.16%)	4 (0.08%)
	Total	33 (1.34%)	29 (1.18%)	62 (1.26%)
3rd Dose (Day 90)	Lost to follow-up	0	1 (0.04%)	1 (0.02%)
	Total	0	1 (0.04%)	1 (0.02%)
Phone call visit 1	Withdrawal of consent by Parent for any reason	1 (0.04%)	2 (0.08%)	3 (0.06%)
	Lost to follow-up	3 (0.12%)	5 (0.20%)	8 (0.16%)
	Other	6 (0.24%)	6 (0.24%)	12 (0.24%)
	Total	10 (0.41%)	13 (0.53%)	23 (0.47%)
Phone call visit 2	Withdrawal of consent by Parent for any reason	2 (0.08%)	2 (0.08%)	4 (0.08%)
	Lost to follow-up	7 (0.28%)	7 (0.28%)	14 (0.28%)
	Other	4 (0.16%)	4 (0.16%)	8 (0.16%)
	Total	13 (0.53%)	13 (0.53%)	26 (0.53%)
Phone call visit 3	Withdrawal of consent by Parent for any reason	8 (0.32%)	1 (0.04%)	9 (0.18%)
	Lost to follow-up	0	5 (0.20%)	5 (0.10%)
	Other	2 (0.08%)	2 (0.08%)	4 (0.08%)
	Total	10 (0.41%)	8 (0.32%)	18 (0.36%)
Phone call visit 4	Withdrawal of consent by Parent for any reason	2 (0.08%)	2 (0.08%)	4 (0.08%)
	Lost to follow-up	2 (0.08%)	1 (0.04%)	3 (0.06%)
	Other	0	1 (0.04%)	1 (0.02%)
	Total	4 (0.16%)	4 (0.16%)	8 (0.16%)
Phone call visit 5	Withdrawal of consent by Parent for any reason	2 (0.08%)	3 (0.12%)	5 (0.10%)
	Lost to follow-up	4 (0.16%)	1 (0.04%)	5 (0.10%)
	Other	3 (0.12%)	2 (0.08%)	5 (0.10%)
	Total	9 (0.36%)	6 (0.24%)	15 (0.30%)
Total	Withdrawal of consent by Parent for any reason	68 (2.76%)	63 (2.55%)	131 (2.65%)
	Lost to follow-up	30 (1.22%)	40 (1.62%)	70 (1.42%)
	Other	15 (0.61%)	20 (0.81%)	35 (0.71%)
	Total	113 (4.58%)	123 (4.98%)	236 (4.78%)

The total mean length of the study period from the first dose to the end of follow-up was approximately 21 months for both treatment groups. There was no difference in the length of the study period between sites.

The total mean length of the follow-up period from the last dose to the end of follow-up was approximately 18 months for both treatment groups (Table 3).

Table 3 Length of follow-up period. ITT population

			Number of days from last dose to end of follow-up						
		N	*Mean*	*Std*	*Min*	*Q1*	*Median*	*Q3*	*Max*
Total	*Vaccine*	2468	556.8	121.3	1	490	618	638	731
	Placebo	2468	554.7	126.5	0	490	617	638	733
Doses given									
Day 1 dose only	*Vaccine*	56	248.8	287.1	1	28	110	612.5	731
	Placebo	72	223.9	270.9	6	27.5	105.5	366	733
Day 1 and day 90 dose	*Vaccine*	91	574	88.44	333	474	629	639	644
	Placebo	85	571.4	98.5	134	532	626	639	645
Day 1 and day 15 dose	*Vaccine*	137	487.2	260.9	31	186	629	711	719
	Placebo	134	486	262.4	39	151	619	713	721
Day 1, day 15 and day 90 dose	*Vaccine*	2184	568.4	84.21	49	504	619	638	651
	Placebo	2177	569.2	83.78	0	504	621	638	662

4 EFFICACY EVALUATION

4.1 Data Sets Analysed

The overview of populations in this this study are summarised by treatment and as a total number of subjects in Table 4. A total of 4123 (83.5 %) of the dosed subjects were included in the Per Protocol (PP) population.

Table 4 Overview of Populations

Population [Statistic]	*Vaccine*	*Placebo*	*Total*
Enrolled population [N]			5253
Safety population [N]	2474	2462	4936
Intention-to-treat (ITT) population [N(%)]	2468 (100%)	2468 (100%)	4936 (100%)
Per protocol (PP) population [n(%)]	2041 (82.7%)	2082 (84.4%)	4123 (83.5%)
Per protocol population for first two doses [n(%)]	2169 (87.9%)	2210 (89.5%)	4379 (88.7%)
Immunological analysis population [n(%)]	69 (2.8%)	53 (2.1%)	122 (2.5%)
Reactogenicity cohort / cohort 1 [n(%)]	175 (7.1%)	175 (7.1%)	350 (7.1%)

4.2 Demographic and other Baseline Characteristics

4.2.1 Demographics

In the ITT population there were a similar total number of males and females (N=2437, 49.4% males and N= 2499, 50.6% females) with similar numbers of males and females in the vaccine group (1191 males and 1277 females) and placebo group (1246 males and 1222 females). There was also an even distribution of males and females recruited at all sites.

The sites that recruited the most subjects were Farafenni Hospital / Farafenni RCH Clinic (N=1441, 29.2%) and Kerewan Health Centre (N=1416, 28.7%).

Most of the subjects were of Mandinka (N=1819, 36.9%) Wolof (N=1540, 31.2%) or Fula (N=1197, 24.3%) ethnic origin with no difference between the vaccine group and placebo group.

The mean age of the subjects at the first dose was 11.5 months ranging from 6 months to 21.5 months with no difference between the age in the vaccine group and placebo group or any site differences. There were more subjects in the younger age group (<12 months) with the majority of subjects aged 6-9 months at first dose than in the 12 months and over age groups.

The mean height of the subjects at first dose was 71.5cm ranging from 55.9cm to 85cm and the mean body weight of the subjects at first dose was 8.3kg ranging from 4.6kg to 14.6kg with no difference between the vaccine group and placebo group. The mean weight-for height z-score at first dose was -0.4 ranging from -3.6 to 6 with no difference between the vaccine group and placebo group.

The mid upper arm circumference of the subjects at first dose was 13.9cm ranging from 10.4cm to 19.2cm with no difference between the vaccine group and placebo group or site differences.

In summary the mean age of the mother was 26.9 years ranging from 16 to 50 years and the mean age of the father was 39.8 years ranging from 20 to 81 years with no difference in age range between the treatment groups or between sites.

Most of the parents had no formal schooling (66.4% of the mothers and 59% of the fathers) with no difference between the treatment groups.

The most common occupation for the fathers was a farmer (45.7%) and the most common occupation for the mothers was a housewife (60.8%) with no difference between the treatment groups.

In summary the mean number of people living regularly in the child's household in the past 6 months was 14.9 ranging from 2 to 75 which was similar for the vaccine and placebo treated groups and for all sites.

The mean number of people sleeping regularly in the child's household for the past 6 months was 14.9 (ranging from 1 to 145 which was slightly less in the vaccine treatment group where the range was from 1 to 94. There were a mean number of 5.6 rooms for sleeping ranging from 0 to 44 rooms which was similar for the vaccine treatment group and placebo treatment groups.

The predominant flooring in the homes was cement (63.2%) and the predominant household possessions were telephones (95.7%), agricultural land (74.0%) and animal drawn carts (63.3%). Wood was the main cooking fuel (93.8%) and fowl and rodents were the most common animals living in the compound (79.6% and 74% respectively). There was no difference between the socio-demographics observed for the vaccine and placebo treatment groups and for all sites.

The main source of drinking water was a public tap (61%) and the facility most used to dispose of human faecal waste was a pit latrine (54%). There was no difference between socio-demographics observed for the vaccine and placebo treatment groups and for all sites.

4.2.2 Demographics conclusion

All demographic parameters were similar between the vaccine and placebo treated groups suggesting treatment groups are comparable.

4.2.3 Medical History

In the ITT population, medical history was recorded for 466 (9.4%) subjects and the most prevalent medical conditions were diarrhoea (n=138, 2.8%), pyrexia (n=106, 2.1%), general infections and infestations (n=106, 2.1%) and malnutrition (n=83, 1.7%). There was no difference in the medical history of the vaccine treated, and placebo treated groups.

4.2.4 Concomitant Medication

The most frequently taken concomitant medication were analgesics, antibacterials for systemic use, anti-diarrhoeals, intestinal anti-inflammatory / anti-infective agents, mineral supplements and vaccines. Similar concomitant medication was taken in both the vaccine and placebo treatment groups.