Two Randomized Trials of Effect of Live Attenuated Influenza Vaccine on Pneumococcal Colonization

## Authors

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# Author contributions

JR, DW, ADHW, SBG, NF, DMF designed the trial.

JR, ADHW, HH, CH, RR, CL, HA, SRZ, VC, LL, KP, IW, ELS, BM, JB, HB conducted the trial according to the study protocol.

SP, EN, EG, EM, SPJ, BC, CS, JRe, JFG participated in site work including laboratory processing, data collection, and challenge preparation.

JR, WAASP, SP, EN, EG, EM, SPJ, BC, CS, MLC, KA, AK, DW, MJM, DB, NF, DMF contributed to laboratory analysis, data interpretation, statistical analysis, and literature search.

JR, WAASP, MJM, DMF drafted the report.

All authors contributed to critical review of the report.

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# To the editor,

The human nasopharynx is frequently colonized by *Streptococcus pneumoniae* (the pneumococcus), serving as the reservoir for transmission, a state which necessarily precedes invasive pneumococcal infection. Influenza infection increases pneumococcal colonization density and dysregulates host immune responses, increasing the risk of secondary bacterial pneumonia and death.(1-3)

Live Attenuated Influenza Vaccine (LAIV) nasal spray has been used in the US since 2003, and has reduced severe influenza disease in the UK since its introduction in 2013 to the national paediatric immunisation program. In mice, LAIV vaccination increases the density and duration of pneumococcal colonization,(2) and rates of otitis media. In children, LAIV is associated with increased rates and density of bacterial colonization.(4) Whilst LAIV is safe and not associated with increases in pneumococcal disease, these data suggest it could increase pneumococcal transmission to susceptible individuals.(5)

We therefore undertook two trials (EudraCT 2014-004634-26) using an established human challenge model to evaluate LAIV effects on the dynamics of pneumococcal colonization. Trial details and results have been deposited on a preprint server.(6) Extensive immunological investigation to accompany these clinical data are published.(7) Healthy non-smoking volunteers, aged 18-50 consented to double-blinded, randomised, placebo-controlled trials reflecting alternative scenarios: 1) *Immunisation-first* – LAIV precedes nasopharyngeal inoculation with pneumococcus by three days; 2) *Colonization-first* – LAIV is administered three days after colonization with pneumococcus. Participants, who were uncolonized at baseline, randomly received either intervention (nasal LAIV, AstraZeneca, UK, paired with intramuscular placebo of normal saline), or control (nasal placebo normal saline paired with intramuscular Influenza Vaccination, Fluarix Tetra, GlaxoSmithKline, UK) with concealment by blindfolding.

Participants were all inoculated with *Streptococcus pneumoniae* serotype 6B strain BHN418 (80,000 colony forming units per nostril) in 0.1ml solution.(8) “Colonization positivity” was determined by serial nasal washes and defined by detection of serotype 6B by culture at programmed time point from 2 to 29 days.(8, 9) In parallel, PCR detection of pneumococcal *lytA* was performed. In the immunisation-first study, LAIV vaccination preceded pneumococcal inoculation by 3 days (primary endpoint: colonization rate); this order was reversed for the colonization-first study (primary endpoint: area under the curve of bacterial density between days 2 and 14). Results are presented as modified intention to treat, excluding those who did not receive immunisation or inoculation per protocol, or did not complete follow-up. Generalised linear models compared colonization positivity, duration of colonization, and AUC bacterial density, with generalised estimating equations employed for comparison at multiple time points. Full methodological and other details are available online.(6)

In the “immunisation first” study (Figure 1), we enrolled 202 participants of whom 130 were inoculated, and 117 analysed (n=55 LAIV, n=62 control, overall mean age 20 [range 18-48] and 58% female). Pneumococcal colonization rates were similar in LAIV participants and controls (25/55 [45.5%] vs 24/62 [38.7%], OR=1.32, p=0.46), although the LAIV-treated group had consistently yet non-significantly higher rates at each time point. PCR detection rates were significantly higher in LAIV versus control at day 2 (33/55 [60.0%] vs 25/62 [40.3%], OR=2.22, p=0.03). Median duration of colonization was not different between groups by conventional microbiology (22 days [IQR 22-29] and 22 days [IQR 14-29] in LAIV and control respectively, p=0.09) or PCR (median 22 days [IQR 7-29] LAIV vs 14 days [IQR 7-22] control, p=0.45). Mean colonization densities were consistently increased in the LAIV group, with statistical significance at d9 representing a 10-fold (1 log10) increase in colonization density in the LAIV group (2.82±1.78 vs 1.81±1.39 log10 titers, p=0.03, Fig. 1). PCR results showed the same pattern, with significantly higher densities in LAIV group at day 2 (p=0.03).

Four participants with laboratory confirmed other viral infections (3 influenza B in control arm, 1 rhinovirus in LAIV arm), had among the highest bacterial densities of their cohorts. Amongst pneumococcal-colonized individuals, the AUC of colonization density was higher in the LAIV group versus controls, with borderline statistical significance in the d2-d14 interval (p=0.05), and reaching statistical significance after exclusion of participants who had nasal swab PCR evidence for concurrent wild-type viral illness (3 influenza B in control arm, 1 rhinovirus in LAIV arm, data not shown, p=0.03), after presenting with symptoms of illness.

In the “colonization first” study (Figure 2), 316 participants consented, 206 were screened, and 163 participants entered the modified ITT analysis (n=73 LAIV, n=90 control, overall mean age 20 [range 18-46], 55% female). Data from seventeen (10%) participants were excluded due to non-study-serotype *S. pneumoniae* colonization. AUC colonization densities for each time period were consistently lower in the LAIV group, though not statistically significant (p=0.11 for d2-14 primary endpoint, Fig 2). By PCR, significantly lower AUC was evident in LAIV vs controls for d2-27 (p=0.03).

Rates of colonization were not different between LAIV and control groups by conventional microbiology (36/73 [49.3%] vs 45/90 [50.0%] respectively, OR=0.97, p=0.93). Median colonization duration not different (21 vs 27 days, p=0.17) by conventional microbiology, although lower in the LAIV group by PCR (14 days vs 27 days, p=0.001).

There were no Serious Adverse Events related to the intervention in either study.

In the largest-to-date trial involving a controlled human co-infection model, we have studied for the first time the impact of co-infection of live viral vaccine and bacterial pathogen. Immunological parameters have been separately reported.(7)

Antecedent LAIV administration caused modest but significant transient effects on pneumococcal colonization, in keeping with a paediatric RCT showing an increased pneumococcal density following LAIV.(2) In our study, the inverse scenario (LAIV following pneumococcal colonization) was associated with reduced colonization density and colonization rates at day 27, decreased AUC, and earlier bacterial clearance.

Our model, consistent with murine co-infection disease models, reinforces that the precedence of pathogen exposure might determine disease outcome: pneumococcal infection following influenza might exacerbate disease, whereas pneumococcus infection preceding influenza may reduce mortality.(10) We used complementary methods for bacterial detection: while PCR is more sensitive and could detect DNA in the absence of viable pathogen, the persistence beyond two days suggests lower-density colonization unmeasurable by culture.

Studies were limited by size, and evaluation of a single pneumococcal serotype in healthy adults likely to have neutralizing influenza antibodies. Any LAIV effect in children may therefore be more pronounced due to lower antibody titres, increased viral shedding, and higher natural rates of pneumococcal colonization acquisition. Future vaccine studies should evaluate the effect on pathogens not directly targeted by the vaccine, including their onward transmission.

# Competing interests

The authors confirm that they have no competing interests.

# Data availability statement

Streptococcus pneumoniae BHN418 sequence is available GI:557376079 (<https://www.ncbi.nlm.nih.gov/nuccore/557376079>).

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# Figure 1 legend

**Figure 1. “Immunisation first”: LAIV precedes nasopharyngeal inoculation with pneumococcus - effect on colonization dynamics**

LAIV vaccination given at day -3. Density dynamics following pneumococcal inoculation (on day 0) are calculated from classical microbiology (log10[CFU/ml+1]. Mean density of *S. pneumoniae* for each nasal wash time point amongst participants in whom serotype 6B was detectable at any point. Bars represent standard errors. Inset: area under the curve (AUC) of density-time from day 2 to day 14 (box plot of median with IQR, with whiskers at 1.5x the IQR).

# Figure 2 legend

**Figure 2. “Colonization first”: LAIV is administered to those already inoculated with pneumococcus - effect on colonization dynamics**

Experimental inoculation to pneumococcus performed at day -3. Density dynamics following LAIV vaccination or control (on day 0) are calculated from classical microbiology (log10[CFU/ml+1]. Mean density of *S. pneumoniae* for each nasal wash time point amongst participants in whom serotype 6B was detectable at any point. Bars represent standard errors. Inset: area under the curve (AUC) of density-time from day 2 to day 14 (the primary endpoint, box plot of median with IQR, with whiskers at 1.5x the IQR).