Effectiveness of the quadrivalent live attenuated influenza vaccine against influenza-related hospitalisations and morbidity among children aged 2 to 6 years in Denmark: a nationwide cohort study emulating a target trial

Helene Kildegaard, Lars Christian Lund, Anton Pottegård, Lone Graff Stensballe

Summary

Background Scant evidence exists on the real-world effectiveness of quadrivalent live attenuated influenza vaccines (LAIV-4) in younger children. We aimed to assess the real-world effectiveness of LAIV-4 against influenza-related hospital contacts and admission and morbidity.

Methods Using nationwide Danish health-care registries, we designed a cohort study that emulates a target trial, comparing LAIV-4 to no vaccination in children aged 2–6 years. Eligible children vaccinated from Oct 1, 2021, to Jan 15, 2022, were matched to unvaccinated controls in a 1:1 ratio according to demographic characteristics and risk groups for influenza, and followed-up until May 31, 2022. Primary study outcomes any hospital contact for influenza and influenza-related hospital admissions more than 12 h in duration, while hospital admission for respiratory tract infections, or for wheezing or asthma, and antibiotic prescriptions were evaluated as secondary outcomes. We estimated incidence rate ratios (IRRs) and 95% CIs using Poisson regression for each outcome. Vaccine effectiveness was calculated as 1−IRR.

Findings Among 308 520 Danish children aged 2–6 years, 95 434 vaccinated children were matched with 95 434 unvaccinated children who acted as controls. Receipt of LAIV-4 compared with no vaccination was associated with a reduced IRR of 0·36 (95% CI 0·27 to 0·46) and estimated vaccine effectiveness of 64·3% (53·6 to 72·6) against influenza-related hospital contacts (76 *vs* 210 events). The corresponding IRR and vaccine effectiveness against influenza-related hospital admissions were 0·63 (0·38 to 1·05) and 36·9% (–5·2 to 62·1; 24 *vs* 38 events), respectively. LAIV-4 was not associated with reductions in admission rates for respiratory tract infections (IRR 1·14, 95% CI 0·94 to 1·38), wheezing or asthma (1·04, 0·83 to 1·31), or antibiotic prescriptions for respiratory tract infections (0·97, 0·93 to 1·00). Vaccine effectiveness assessed across risk groups for influenza showed similar effectiveness in children with and without coexisting risk factors for severe influenza.

Interpretation LAIV-4 offered moderate protection in younger children against influenza-related hospital contacts during a season dominated by influenza A(H3N2); however vaccination was not associated with reductions in secondary outcomes. This real-world study thereby supports trial evidence of moderate vaccine effectiveness of LAIV-4 against influenza-related outcomes when implementing broad vaccination schedules in younger children.

Funding Beckett-Fonden

Lancet Child Adolesc Health 2023

Published Online
October 25, 2023
https://doi.org/10.1016/S2352-4642(23)00225-0

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Introduction

Influenza prevention strategies remain a topic of ongoing debate for the paediatric population. In young children, an estimated 900 000 hospitalisations and 35 000 child deaths are caused by influenza-related acute lower respiratory infection each year.[1] Vaccination is a potentially effective method to reduce childhood morbidity from influenza and might offer indirect protection for vulnerable groups through reduction in influenza transmission.[2] National vaccination schedules for influenza generally include children at high risk of severe influenza, eg, children with chronic disease or immunodeficiencies. Conversely, policies for healthy children vary from omission of healthy children from vaccination programmes to age-based schedules targeting toddlers and preschool children (eg, Spain and Finland), while only few countries, including the USA and Canada, have extended their schedules to include all children older than 6 months.[3,4]

Many countries recommend use of live attenuated influenza vaccines (LAIV) for children aged 2 years or older. The trivalent LAIV (LAIV-3) was licensed in the USA in 2003, and approved for use in the EU in 2011. The current quadrivalent formulation of LAIV (LAIV-4) obtained licensing during 2012–13, subsequently replacing LAIV-3. LAIV are administered intranasally, and stimulate broad immune responses,

Research in context

Evidence before this study

Estimates of real-world effectiveness of influenza vaccines in children are generally lower than the efficacies reported in randomised trials, and there are insufficient data from randomised trials on the protection from influenza vaccines on influenza-related hospitalisations and other more severe outcomes of influenza in children. In addition, effectiveness can vary substantially from season to season, by influenza vaccine type, and by the degree of match between vaccine strain and circulating influenza variant. We searched PubMed for articles published from database inception through April 25, 2023, without language restrictions, using the search terms "influenza vaccination", "live attenuated influenza vaccination" and "children" in combination with terms related to "efficacy", "effectiveness", "antibiotics", and "beneficial OR protective OR secondary effects". When available, we prioritised systematic reviews and meta-analyses, and searched through reference lists to identify other relevant articles. Few studies have evaluated the effectiveness of quadrivalent live attenuated influenza vaccines in young children, and there is a paucity of data regarding secondary derivative effects.

Added value of this study

Compared with previous studies, this observational study emulating a target trial comparing vaccination with quadrivalent live attenuated influenza vaccines (LAIV-4) to no vaccination, showed higher, but only moderate, effectiveness of LAIV-4 against influenza-related hospital contacts during a first-time vaccination schedule targeting healthy children and a season dominated by influenza A(H3N2). The study adds evidence on moderate effectiveness of LAIV-4 against more severe influenza, measured by hospital admissions of at least 12 h in duration. The study also demonstrated substantially better protection in children vaccinated with two doses of LAIV-4, and finds similar vaccine effectiveness across age groups and risk groups for a severe course of influenza. On a population level, vaccination was not associated with secondary effects of vaccination, with similar rates of admissions for respiratory tract infections, or for wheezing or asthma, and rates of antibiotic prescription fills for respiratory tract infections among vaccinated and unvaccinated children.

Implications of all the available evidence

This study adds to the previous evidence by finding suboptimal effectiveness of vaccination with LAIV-4 in younger children, and suggests restricted protection against secondary outcomes. Real-world effectiveness of LAIV-4 was higher against influenza A(H3N2) than previously reported, possibly reflecting increased effects of LAIV-4 due to low pre-existing influenza immunity in the children following the COVID-19 pandemic. Future studies should determine the ability of LAIV-4 to prevent life-threatening influenza illness, investigate the cross-seasonal durability of LAIV-4, optimise vaccination schedules, and focus on improving influenza vaccine technologies.

including cellular, humoral, and mucosal responses.[5] Randomised clinical trials have also shown higher acceptance rates and superior efficacy of LAIV compared with inactivated influenza vaccines,[6,7] leading to their initial preferential use in children in many countries, including Canada and the UK. Observational studies have, however, shown mixed effectiveness of LAIV against influenza-related hospitalisations depending on setting and circulating strain,[8–10] resulting in different vaccine recommendations among nations. Some countries, including the UK and France, continue to recommend LAIV for the paediatric population, while other countries, such as the USA and Canada, use LAIV and inactivated influenza vaccines interchangeably. Existing evidence is largely based on studies of LAIV-3, and there is a paucity of data against more severe influenza outcomes and derivative effects of influenza vaccination, including prevention of asthma exacerbations and secondary infections caused by exposure to influenza virus in the community.[11,12]

In this study we describe vaccine uptake during implementation of an influenza vaccination schedule for healthy children aged from 2–6 years in Denmark. We further aimed to assess the real-world effectiveness of LAIV-4 against influenza-related hospital contacts and hospital admissions during the 2021–22 season, which was characterized by low pre-existing influenza immunity in younger children following the COVID-19 pandemic. Since influenza vaccination would be assumed to also protect against respiratory infections and wheezing caused by influenza virus, but remain undiagnosed as no diagnostic test was carried out, we also evaluated vaccine protection against secondary outcomes, including hospital admissions for respiratory tract infections, wheezing and asthma, and antibiotic prescriptions for respiratory tract infections.

Methods

Study design and participants

In this population-based cohort study, we collated data on all children aged 2–6 years in Denmark who were eligible for an influenza vaccination during the 2021–22 influenza season, using individual-level linked data from Danish nationwide health-care registries and databases. We describe the uptake of the influenza vaccine and assess differences in characteristics among vaccine recipients and non-recipients. Using target trial principles,[13] as applied in studies of the effectiveness of COVID-19 vaccines,[14] we designed this observational study to emulate a target trial to estimate the effectiveness of LAIV-4 against influenza-related outcomes. To explore the effect of our choice of method, we repeated the

analysis using a conventional cohort design and the test-negative design commonly used for evaluation of influenza vaccine effectiveness.[15]

The study included data from six Danish nationwide registers: The Danish Civil Registration System, the National Health Service Registry, the National Patient Registry, the National Prescription Registry, the Medical Birth Registry, and the Danish Microbiology Database.[16,17] The study was registered at the repository of the University of Southern Denmark (11.106), and data access was approved by the Danish Health Data Authority (FSEID-00005038).

Procedures

Before the 2021–22 influenza season only high-risk individuals were eligible for childhood influenza vaccination in Denmark. However, starting in the autumn of 2021, the vaccination schedule was expanded to all children aged 2–6 years, who were offered influenza vaccination free of charge from Oct 1, 2021, to Jan 15, 2022. Two doses of LAIV-4 were recommended, unless specific contraindications were present, in which case children were offered vaccination with quadrivalent inactivated influenza vaccines (IIV-4). Most vaccinations were carried out by general practitioners who received reimbursement for these vaccinations with a specific code (8926), registered in the Danish National Health Service Register along with the date of vaccination.

In Denmark, influenza tests performed at general practitioners and in hospitals settings are analysed by real-time PCR (RT-PCR) and registered within the Danish Microbiology Database.[17] According to national guidelines, only people belonging to high-risk groups presenting with influenza-like illness are tested in primary care. Otherwise, testing for influenza is restricted to hospital settings, where testing for influenza is recommended in individuals with influenza-like illness or lower respiratory symptoms during the influenza season.[18]

All children with residence in Denmark, born from Jan 16, 2015, to Jan 15, 2020, were eligible for vaccination free of charge. For baseline characterisation of the eligible cohort, vaccination status was determined on Jan 15, 2022, which according to national guidelines served as the last day for enrolment in the influenza vaccination schedule. The following potential determinants of vaccine acceptance were included based on existing literature: parental characteristics (maternal age at birth, parental chronic diseases, maternal smoking during pregnancy), demographics (age, sex, birth order, number of siblings, residential area, immigration status), medical history (prematurity, chronic disease, recent admission to hospital for wheezing or respiratory tract infections), and previous acceptance of vaccines within the Danish childhood vaccination programme. For further definitions of covariates, including International Classification of Diseases 10th edition (ICD-10) codes, and Anatomical Therapeutic Chemical Classification (ATC) codes, see the appendix (pp 4–5).

To assess the effectiveness of LAIV-4, we designed our observational cohort study to emulate a pragmatic trial comparing LAIV-4 with no vaccination for prevention of influenza-related outcomes among children aged 2–6 years. Specifications of each component in the target trial and observational emulation are specified in detail in the appendix (pp 6–7). Eligibility criteria included an age of 2–6 years, continuous residency in Denmark for at least 1 year before inclusion, no previous influenza vaccination, and no contraindication for LAIV-4. To emulate willingness to participate in a clinical vaccine trial we only included children who had previously participated in the Danish childhood vaccination programme. Children were recruited between Oct 1, 2021, and Jan 15, 2022.

The intervention of interest was receipt of LAIV-4, and we separately analysed the effect of only one dose, or two doses of LAIV-4 as specified below. For each week of the study period, we identified eligible individuals who were unvaccinated as of the first day of the week (Monday). All newly vaccinated children within that week were then matched in a 1:1 ratio without replacement to unvaccinated children who had not previously been matched as a control pair. To ensure balance of important characteristics across groups, vaccinated children were matched to unvaccinated children according to birth quartile, sex, residential region, immigration status, and a number of risk factors for severe influenza as defined by national guidelines. Each matched pair was followed from 14 days after the enrolment date until occurrence of an outcome event—emigration, death, or end of follow-up on May 31, 2022—whichever came first. Both members of a matched pair were censored if the unvaccinated child was vaccinated during follow-up, at which point the previously unvaccinated child could instead be included in the vaccinated cohort. Such censoring was applied to maintain comparability of the two groups with regards to matching factors.

Outcomes

The two primary outcomes were any hospital contact for influenza and influenza-related hospital admissions more than 12 h in duration, with hospital admissions reflecting increased severity of disease. Hospital contacts and admissions were categorised as influenza-related if children had a positive influenza test swabbed up to 4 days before or during hospitalisation, or had a discharge diagnosis of influenza. Secondary outcomes were hospital admissions for respiratory tract infections, wheezing or asthma, and antibiotic prescription fills for respiratory tract infections. For secondary outcomes, we allowed each individual to have repeated outcomes. For associated ICD-10, ATC code, and other details on outcome definitions, see the appendix (p 8).

Statistical analysis

Among the entire cohort of Danish children eligible for influenza vaccination, we charted the cumulative

See Online for appendix

proportion of children who received an influenza vaccine from Oct 1, 2021, to Jan 15, 2022. We used descriptive statistics to characterise vaccine recipients and non-recipients and assessed differences in vaccine acceptance using standardised mean differences, with a difference of 0·1 or less considered negligible.[19]

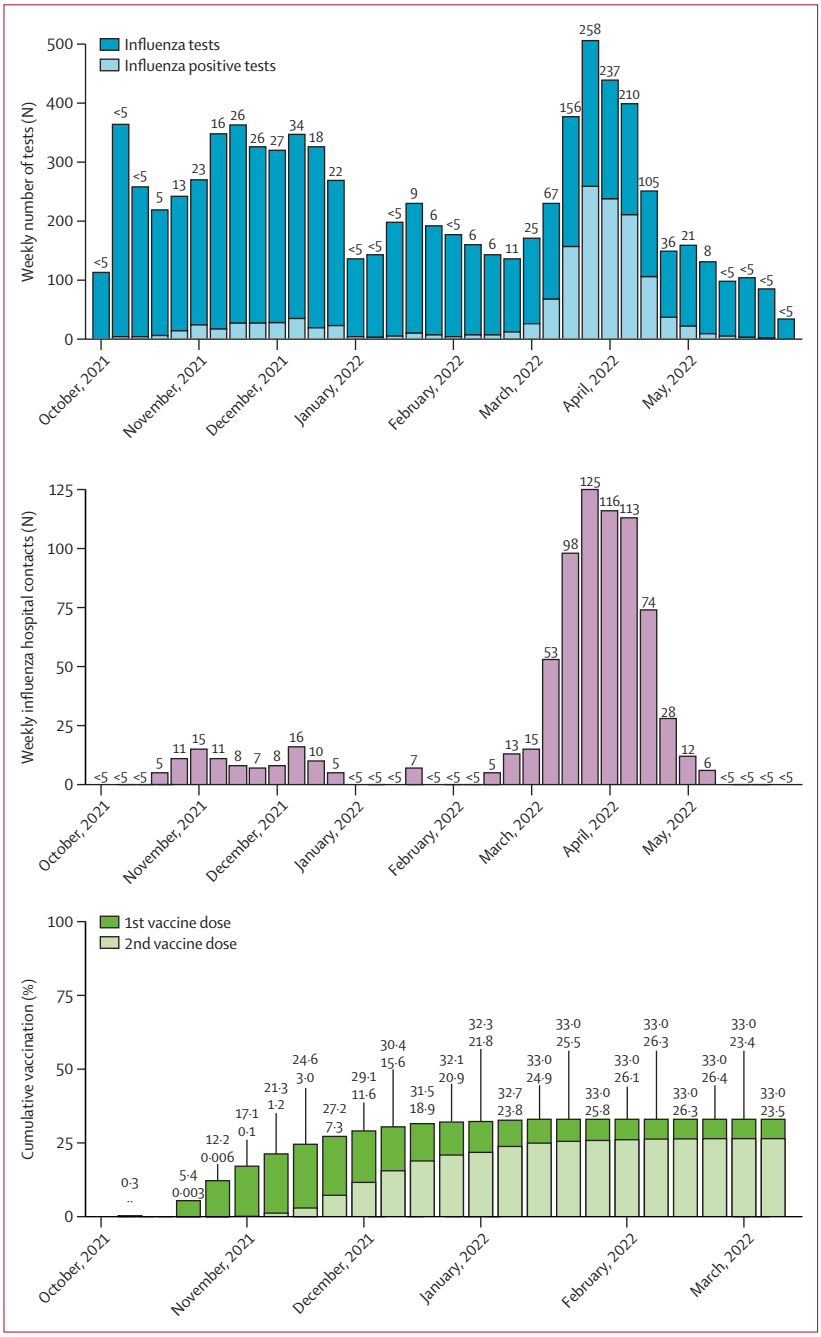

Figure 1: **Overview of the influenza epidemic 2021–22 in Danish children aged 2–6 years.**
(A) Weekly number of positive and negative real-time PCR tests for influenza. Numbers above bars indicate positive tests. (B) Weekly number of children with any hospital contact with influenza. Numbers above bars indicate hospital contacts. (C) Cumulative proportion of children vaccinated against influenza. Numbers above bars indicate cumulative proportions of first (top) and second (bottom) doses.

In the target trial emulation we constructed cumulative incidence curves for influenza-related outcomes. For the main analysis we evaluated vaccine effectiveness against study outcomes from day 14 after receipt of the first dose of LAIV-4 until the end of the influenza season, defined as May 31, 2022, regardless of whether children subsequently received their second dose as planned. In secondary analyses we evaluated vaccine effectiveness after only one vaccination dose, censoring the matched pair if the vaccinated child received a second dose during follow-up, and after the full vaccination scheme of two doses. In both analyses, follow-up started on day 14 after vaccination and for the matched unvaccinated child on the corresponding date. We estimated incidence rate ratios (IRRs) and incidence rate differences (IRDs) for each outcome with 95% CIs using Poisson regression. For influenza-related hospital contacts and admissions, we further estimated the vaccine effectiveness as 1−IRR for each outcome. None of the covariates used in the estimation of vaccine effectiveness had missing data. Subgroup analyses were conducted stratified by age group, and whether or not children belonged to high-risk groups for severe influenza.

Several sensitivity analyses were performed to test the robustness of our findings. First, to test the effect of our main design choice with sequential inclusion strategy and exact matching and censoring criteria, we analysed the data using a traditional cohort design, assigning unvaccinated comparators a random index date from the distribution of vaccination dates among the vaccinated cohort to account for temporality. Inverse probability of treatment weights were used to adjust for confounding. Second, we estimated vaccine effectiveness against influenza-related outcomes using a test-negative design, restricting analyses to all eligible children tested for influenza from Oct 1, 2021 to May 31, 2022. As post-hoc analyses, we repeated the main analysis with no censoring of the vaccinated case if the unvaccinated child was vaccinated during follow-up. We also extended the window following vaccination where children could not by design experience the outcome from days 14–30 due to a risk of false-positive influenza samples following LAIV-4. Details on sensitivity analyses are provided in the appendix (p 3). All analyses were performed using STATA MP, version 17.1.

Role of the funding source

The funders had no role in study design, acquisition of data, analysis, interpretation, or preparation of the manuscript.

Results

Between Oct 1, 2021, and Jan 15, 2022, 308 520 Danish children were potentially eligible for influenza vaccination during the 2021–22 season. The timing of the influenza epidemic and vaccine uptake are presented in figure 1. A total of 8377 influenza RT-PCR tests (from 7210 children)

were analysed, and 1396 were positive, yielding an overall season positive percentage of 16·7%. Low numbers of influenza cases were observed during the autumn and early winter, but started to increase from mid-February, 2022, peaking in late-March, 2022. By the end of the season, 784 children had had an influenza-related hospital contact. Overall, 101 885 children (33·0%) of 308 520 were vaccinated, and 81 626 (26·5%) received the full vaccination scheme, consisting of two doses (figure 1). Vaccinated and unvaccinated children resembled each other on most measured covariates (table 1). Vaccinated children did, however, have a slightly lower median age, were more likely to be first-born children, and their mothers were older at birth and had more often refrained from smoking during pregnancy, compared with unvaccinated children. There were no differences among vaccinated and unvaccinated children in terms of medical history or number of risk factors for severe influenza, but vaccinated children had higher prior participation in the Danish childhood vaccination programme than their unvaccinated peers (table 1).

After applying exclusion criteria and matching in the target trial emulation, 95 434 vaccinated and 95 434 unvaccinated children were included in the final study population (figure 2). The groups had identical distributions of matching criteria and were comparable in terms of individual risk factors for severe influenza (table 1). The incidence of influenza-related hospital contacts was low, and similar in the vaccinated and unvaccinated group until 120 days follow-up, but

	Before matching			After matching		
	Vaccinated (n=101 885)	Unvaccinated (n=206 635)	SMD	Vaccinated (n=95 434)	Unvaccinated (n=95 434)	SMD
Demographics						
Median age, years (IQR)*	4 (2–5)	4 (3–5)	0·11	4 (3–5)	4 (3–5)	0·02
Sex						
Female*	49 393 (48·5%)	100 534 (48·7%)	0·00	46 506 (48·7%)	46 506 (48·7%)	0·00
Male*	52 492 (51·5%)	106 101 (51·3%)	0·00	48 928 (51·3%)	48 928 (51·3%)	0·00
Immigration status*						
First generation	1899 (1·9%)	4581 (2·2%)	0·02	977 (1·0%)	977 (1·0%)	0·00
Second generation	10 598 (10·4%)	27 770 (13·4%)	0·09	9834 (10·3%)	9834 (10·3%)	0·00
Maternal age in years at birth, median (IQR)	31 (28–34)	30 (27–34)	0·17	31 (28–34)	30 (27–34)	0·15
<25	7966 (7·8%)	27 099 (13·1%)	0·17	7363 (7·7%)	11 381 (11·9%)	0·14
25–34	70 908 (69·6%)	138 977 (67·3%)	0·05	66 514 (69·7%)	65 106 (68·2%)	0·03
≥35	23 233 (22·8%)	41 561 (20·1%)	0·07	21 697 (22·7%)	19 179 (20·1%)	0·07
Number of siblings						
0	14 194 (13·9%)	30 591 (14·8%)	0·02	10 764 (11·3%)	11 541 (12·1%)	0·03
1	59 756 (58·7%)	112 957 (54·7%)	0·08	45 839 (48·0%)	46 041 (48·2%)	0·00
≥2	27 935 (27·4%)	63 087 (30·5%)	0·07	38 831 (40·7%)	37 852 (39·7%)	0·02
Maternal birth order of the child						
1	51 026 (50·1%)	90 002 (43·6%)	0·13	48 591 (50·9%)	44 108 (46·2%)	0·09
≥2	46 984 (46·1%)	108 064 (52·3%)	0·12	44 496 (46·6%)	49 129 (51·5%)	0·10
Missing	3875 (3·8%)	8569 (4·1%)	0·02	2347 (2·5%)	2197 (2·3%)	0·01
Maternal BMI at pregnancy onset						
Underweight	978 (1·0%)	2657 (1·3%)	0·03	920 (1·0%)	1118 (1·2%)	0·02
Normal weight	56 296 (55·3%)	114 600 (55·5%)	0·00	53 474 (56·0%)	54 162 (56·8%)	0·01
Overweight or obese	38 667 (38·0%)	75 760 (36·7%)	0·03	36 750 (38·5%)	35 848 (37·6%)	0·02
Missing	5944 (5·8%)	13 618 (6·6%)	0·03	4290 (4·5%)	4306 (4·5%)	0·00
Maternal smoking during pregnancy	6525 (6·4%)	20 811 (10·1%)	0·13	6133 (6·4%)	8955 (9·4%)	0·11
Missing	8962 (8·8%)	19 088 (9·2%)	0·02	7145 (7·5%)	7034 (7·4%)	0·00
Parental chronic disease	13 850 (13·6%)	24 765 (12·0%)	0·05	12 985 (13·6%)	11 767 (12·3%)	0·04
Perinatal history						
Prematurity						
Preterm (28–37 weeks)	6226 (6·1%)	10 775 (5·2%)	0·04	5832 (6·1%)	5224 (5·5%)	0·03
Extremely preterm (<28 weeks)	243 (0·2%)	341 (0·2%)	0·02	176 (0·2%)	150 (0·2%)	0·01
Missing	3979 (3·9%)	8865 (4·3%)	0·02	2445 (2·6%)	2320 (2·4%)	0·01
Low birth weight (<2500 g)	4970 (4·9%)	8719 (4·2%)	0·03	4589 (4·8%)	4164 (4·4%)	0·02
Missing	4150 (4·1%)	9270 (4·5%)	0·02	2601 (2·7%)	2449 (2·6%)	0·01

(Table 1 continues on next page)

	Before matching			After matching		
	Vaccinated (n=101 885)	Unvaccinated (n=206 635)	SMD	Vaccinated (n=95 434)	Unvaccinated (n=95 434)	SMD
(Continued from previous page)						
Medical history						
Risk factors for severe influenza						
Other chronic respiratory diseases	387 (0·4%)	474 (0·2%)	0·03	204 (0·2%)	173 (0·2%)	0·01
Chronic heart disease and major congenital malformations of the heart	1392 (1·4%)	2527 (1·2%)	0·01	1144 (1·2%)	1148 (1·2%)	0·00
Neuromuscular disease	529 (0·5%)	1044 (0·5%)	0·00	439 (0·5%)	437 (0·5%)	0·00
Malignancy, haemotological disease, or immunodeficiency	542 (0·5%)	1061 (0·5%)	0·00	209 (0·2%)	209 (0·2%)	0·00
Type 1 diabetes	86 (0·1%)	121 (0·1%)	0·01	65 (0·1%)	58 (0·1%)	0·00
Metabolic disorders	371 (0·4%)	792 (0·4%)	0·00	307 (0·3%)	332 (0·3%)	0·00
Chronic renal or liver disease	572 (0·6%)	1134 (0·5%)	0·00	491 (0·5%)	511 (0·5%)	0·00
Obesity	180 (0·2%)	373 (0·2%)	0·00	164 (0·2%)	154 (0·2%)	0·00
Number of risk factors for severe influenza*						
0	98 157 (96·3%)	199 620 (96·6%)	0·01	92 577 (97·0%)	92 577 (97·0%)	0·00
1	3433 (3·4%)	6552 (3·2%)	0·01	2755 (2·9%)	2755 (2·9%)	0·00
≥2	295 (0·3%)	463 (0·2%)	0·01	102 (0·1%)	102 (0·1%)	0·00
Other comorbidities						
Asthma	5433 (5·3%)	7385 (3·6%)	0·09	4745 (5·0%)	3531 (3·7%)	0·06
Epilepsy	439 (0·4%)	880 (0·4%)	0·00	390 (0·4%)	364 (0·4%)	0·00
Autoimmune disorders	423 (0·4%)	911 (0·4%)	0·00	385 (0·4%)	394 (0·4%)	0·00
Psychiatric disorders	1553 (1·5%)	3413 (1·7%)	0·01	1470 (1·5%)	1511 (1·6%)	0·00
Hospital admission for wheezing or RTI within the last year	722 (0·7%)	1165 (0·6%)	0·02	633 (0·7%)	540 (0·6%)	0·01
Number of antibiotic prescriptions within the last year						
0	89 179 (87·5%)	183 124 (88·6%)	0·03	82 591 (86·5%)	83 437 (87·4%)	0·03
1	8824 (8·7%)	16 768 (8·1%)	0·02	8970 (9·4%)	8481 (8·9%)	0·02
≥2	3882 (3·8%)	6743 (3·3%)	0·03	3873 (4·1%)	3516 (3·7%)	0·02
Adherence to childhood vaccination program						
Fully vaccinated	80 748 (79·3%)	151 210 (73·2%)	0·14	78 719 (82·5%)	77 045 (80·7%)	0·05
Partially vaccinated	19 724 (19·4%)	49 056 (23·7%)	0·11	16 715 (17·5%)	18 389 (19·3%)	0·05
No vaccination	1413 (1·4%)	6369 (3·1%)	0·11	..	..	..

Data are n (%) unless otherwise stated. Children were categorised as vaccinated if they received at least one vaccination against influenza. For baseline description of children eligible for vaccination, covariates were assessed on Oct 1, 2021. For children included in the target trial emulation, covariates were assessed on the enrolment date. Data on ethnicity and socioeconomic status were not available from our data sources. SMD=standard mean difference. RTI=respiratory tract infection. *Denotes variables included in 1:1 matching.

Table 1: **Demographic and clinical characteristics of children eligible for vaccination with LAIV-4 before and after enrolment in target trial emulation**

afterwards began to diverge, showing more hospital contacts in the unvaccinated group (figure 3A) co-occurring with the increased community spread of influenza (figure 1A). A similar, but smaller, divergence was seen for more severe influenza, measured by hospital admissions of at least 12 h in duration (figure 3B). During follow-up, 76 vaccinated children and 210 unvaccinated children had an influenza-related hospital contact, with an estimated vaccine effectiveness of 64·3% (95% CI 53·6 to 72·6) and a between-group IRD of –33·4 per 10 000 person years (–41·6 to –25·2; table 2). No statistically significant protective effect was observed of receiving only one dose of LAIV-4 (vaccine effectiveness 28·9%, –16·4 to 56·6), while vaccine effectiveness was substantial at 74·3% (64·2 to 81·5) after two doses against any influenza-related hospital contact (appendix p 9). Subgroup analyses showed comparable point estimates for vaccine effectiveness against influenza-related hospital contacts across age groups (appendix p 10) and among children in risk-groups for severe influenza (57·2%, –11·3 to 83·6) and children with no risk factors (64·8%, 53·7 to 73·3), although analyses were limited by low number of events among children with high risk (appendix p 11).

Among children included in the emulated target trial, only 24 vaccinated children and 38 unvaccinated children had an influenza-related hospital admission lasting at least 12 h, yielding an estimated vaccine effectiveness of 36·9% (95% CI –5·2 to 62·1) with a between-group

IRD of –3·5 (–7·3 to 0·3) per 10 000 person years (table 2), but this difference was not statistically significant. The LAIV-4 gave no protection against hospital admissions for other respiratory tract infections or due to asthma or wheezing with IRRs of 1·14 (95% CI 0·94 to 1·38) and 1·04 (0·83 to 1·31) in the main analysis respectively, and it was not associated with clinically relevant reductions in antibiotic prescriptions for respiratory tract infections (IRR 0·97, 0·93 to 1·00; table 2).

Results from sensitivity analyses using a conventional cohort design and the test-negative design are presented in the appendix (pp 12–19). Both designs yielded results similar to the main analysis, although the cohort design returned more precise effect estimates. Vaccine effectiveness of LAIV-4 against hospital admissions of at least 12 h in duration were statistically significant in both sensitivity analyses with effectiveness of 38·1% (95% CI 3·7 to 60·2) and 46·4% (10·6 to 68·0) in the cohort and test-negative design, respectively. Censoring in the main

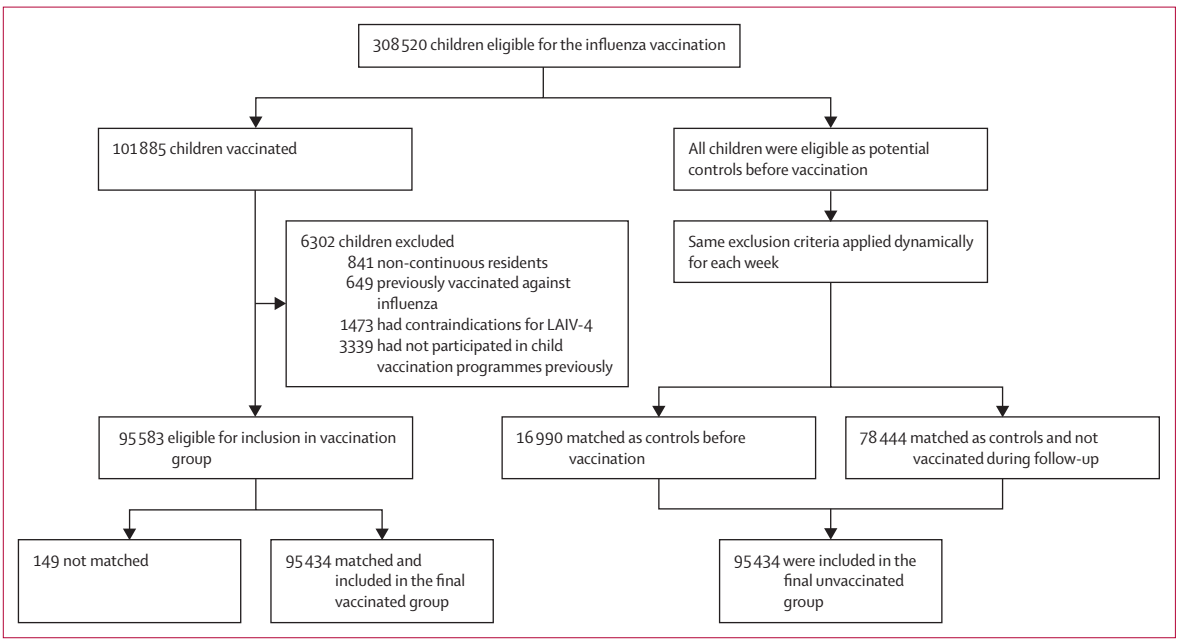

Figure 2: **Study population and enrolment in target trial emulation**
LAIV-4=quadrivalent live attenuated influenza vaccines.

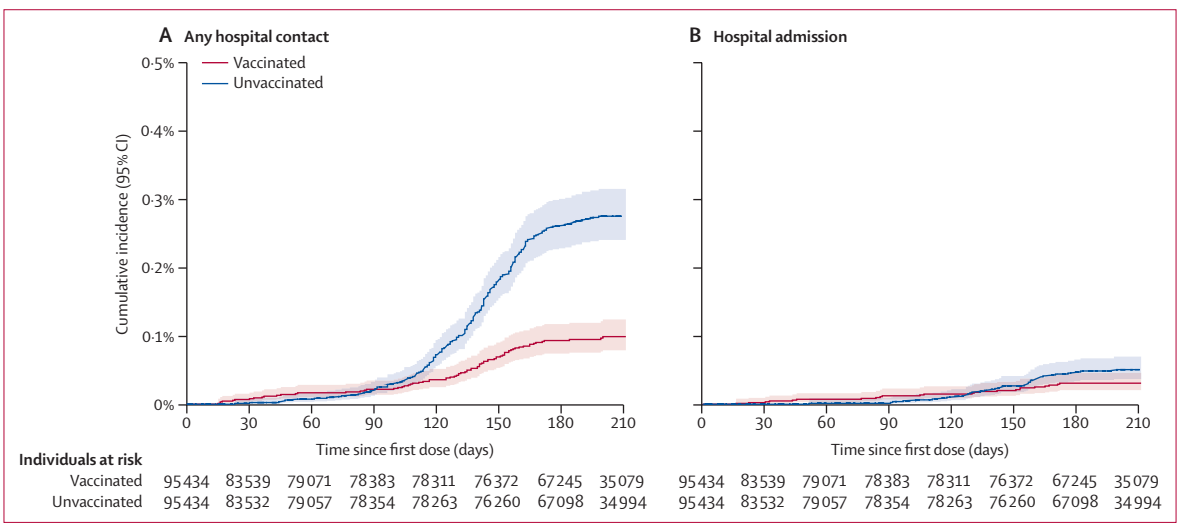

Figure 3: **Cumulative incidence of influenza-related outcomes**
Cumulative incidence of any influenza-related contact (A) and hospital admissions lasting at least 12 h (B) among children aged 2–6 years vaccinated with LAIV-4 compared to matched unvaccinated children. Children were followed from 14 days after receipt of the first dose of LAIV-4 regardless of whether they received a second dose. Shaded areas represent 95% CIs. Note: the y-axis has been expanded. LAIV-4=quadrivalent live attenuated influenza vaccines.

	Number of events		Rate per 10 000 person-years		IRR (95% CI)	IRD per 10 000 person-years (95% CI)	Vaccine effectiveness (95% CI)
	Vaccinated group	Unvaccinated group	Vaccinated group	Unvaccinated group			
Influenza hospital contacts	76	210	18·5	52·0	0·36 (0·27 to 0·46)	–33·4 (–41·6 to –25·2)	64·3% (53·6 to 72·6)
Influenza hospital admissions	24	38	5·9	9·4	0·63 (0·38 to 1·05)	–3·5 (–7·3 to 0·3)	36·9% (–5·2 to 62·1)
Respiratory tract infection hospital admissions	228	200	56·3	49·4	1·14 (0·94 to 1·38)	6·9 (–3·1 to 16·9)	..
Wheezing or asthma hospital admissions	148	142	36·6	35·1	1·04 (0·83 to 1·31)	1·5 (–6·8 to 9·7)	..
Antibiotics for RTI	6412	6628	1584·3	1638·2	0·97 (0·93 to 1·00)	–53·9 (–109·2 to 1·4)	..

Number of events, incidence rates, IRR, IRD, and VE among children vaccinated with LAIV-4 and matched unvaccinated children in target trial emulation. Estimates were adjusted for age, sex, calendar time, residential area, immigration status, and number of risk factors for severe influenza by matching. In this analysis, children were followed up from 14 days after receipt of the first dose of LAIV-4 regardless of whether they received a second dose. IRR=incidence rate ratio. IRD=incidence rate difference. LAIV-4=quadrivalent live attenuated influenza vaccines. RTI=respiratory tract infections.

Table 2: **Vaccine effectiveness against influenza-related and secondary outcomes**

analysis had little change to effect estimates (appendix p 20). Disregarding influenza-related outcomes occurring within the first 30 days of vaccination due to a risk of false-positive influenza test following LAIV-4 vaccination increased vaccine effectiveness to 69·7% (59·9 to 77·2) and 52·7% (17·1 to 73·0) against influenza-related hospital contacts and admissions, respectively (appendix p 21).

Discussion

This nationwide observational study estimated the real-world effectiveness of the LAIV-4 among children aged 2–6 years during the 2021–22 influenza season in Denmark. Vaccine effectiveness of LAIV-4 was 64·3% against any influenza-related hospital contacts and 36·9% against more severe influenza measured by influenza-related hospital admissions at least 12 h in duration. We found no evidence suggesting a protective effect of LAIV-4 against hospital admissions due to other respiratory tract infections, or asthma or wheezing, or against antibiotic prescriptions for respiratory tract infections.

A major strength of the current study is its population-based design, allowing the vaccination status of all children in Denmark and inclusion of all influenza tests performed in Danish hospital settings to be established, thus reducing the risk of selection bias. Due to the nationwide coverage of the Danish health-care registries, we had complete individual-level assessment of previous medical history, and were able to follow-up with study participants throughout the 2021–22 influenza season and across health-care sectors. The moderate vaccine uptake also allowed us to implement target trial emulation methodology to minimise selection bias and potential lack of generalisability inherent to the test-negative design.[14,20]

Our study also has limitations. First, as influenza testing for children in Denmark is primarily recommended in the hospital setting, we cannot capture the complete spectrum of influenza disease, and many infections remain undetected. Outcome misclassification is, however, likely to be non-differential as testing for influenza was recommended for all hospitalised children with influenza-like illness regardless of vaccination status, and was generally combined with testing for COVID-19. Second, evaluation of vaccine effectiveness against hospital admissions was limited by a small number of events, and fewer than five vaccinated children had an intensive care unit admission registered in relation to an influenza-related admission, impeding analysis of vaccine effectiveness against this outcome. Our study only included vaccinations registered by general practitioners, leading to a small underreporting of vaccine coverage, and potential differential misclassification of children with severe chronic disease as unvaccinated if these children were vaccinated in hospital settings. There was also a risk of false-positive influenza samples in the weeks following vaccination with LAIV,[21] potentially explaining the early increase in influenza-related hospitalisations among vaccinated children, and leading to underestimation of vaccine effectiveness as shown in sensitivity analyses. Further, we could not differentiate LAIV-4 and IIV-4 vaccines based on reimbursement codes. We consider this misclassification negligible, as 98·7% of administered vaccines were LAIV-4, and we further excluded children with contraindications for LAIV-4 in analyses of vaccine effectiveness. Finally, as in any observational study, there is a possibility of residual confounding, including healthy vaccinee bias. Vaccinee bias can arise due to unmeasured differences in underlying child health if children in better health conditions are preferentially vaccinated against influenza, and this can lead to potential overestimation of vaccine effectiveness.

The uptake of the Danish national influenza vaccination schedule was substantially lower than for vaccines in the

Danish childhood vaccination programme,[22,23] but was similar to the influenza vaccine uptake described in other countries.[3] In line with previous research, low uptake was seen in children with increasing birth order, those with young mothers, those who had a low uptake of routine childhood vaccines, and children from lower socioeconomic backgrounds.[22,24]

Our estimates of LAIV-4 effectiveness against any influenza-related hospitalisation were higher than those from previous observational studies. Generally, real-world effectiveness of influenza vaccines in children has far from matched the efficacy of 78% (95% CI 59–89) against laboratory-confirmed influenza shown in meta-analysis of randomised trials.[7] No randomised controlled trials have been adequately powered to estimate vaccine effectiveness against hospitalisation or other more severe outcomes of influenza. Observational studies have found that vaccine effectiveness against hospitalisations is lower for live attenuated vaccines than for inactivated vaccines, with meta-analysis effectiveness at 44·3% (30·1–55·7) compared with 68·7% (53·6–79·2).[8] Vaccine effectiveness in children also varies substantially across age groups, settings, and seasons, with the lowest protection against influenza A(H3N2).[8,25] During the 2021–22 season in Denmark, the circulating strain was almost exclusively influenza A(H3N2), mainly clade 3C.2a1b.2a.2, which has changes in the antigenic sites compared with the included influenza A strains that season.[26] Vaccine effectiveness estimates given in this study should, therefore, be interpreted as effectiveness against influenza A(H3N2) despite the absence of virus sequencing at an individual level. Very few studies have evaluated the protection of LAIV-4 specifically against influenza A(H3N2), and published studies found vaccine effectiveness ranging from 0% to 50% in seasons 2014–15 through to 2018–19.[9,10,27–29] Compared with these estimates, this study found higher protection against the influenza A(H3N2)-strain, despite the mismatch between circulating strain and vaccine strain, with a vaccine effectiveness of 64·3% (53·6–72·6). In line with previous research,[25] full vaccination with two doses of LAIV-4 offered a markedly increased protection against influenza-related outcomes compared with partial vaccination in this cohort of children who were previously unvaccinated against influenza. Across all studies, influenza vaccine effectiveness against hospitalisations is, however, only moderate at best, warranting more effective influenza vaccine technologies also for children.

Uncertainty remains about the level of protection afforded by influenza vaccination against more severe outcomes of influenza and influenza-related morbidity. In the current study, we evaluated vaccine effectiveness against hospital admissions lasting at least 12 h to distinguish hospital contacts with only short-term medical care required from inpatient stays. For this outcome, we found low to moderate protection by LAIV-4 with vaccine effectiveness of 33% to 53% depending on the method applied, with sensitivity analyses suggesting stronger protection in children who are fully vaccinated with two doses.

Influenza vaccination may also offer protection against asthma exacerbations and respiratory infections secondary to community spread of influenza.[11,12] We did not find evidence to suggest that LAIV-4 confers protection against admission for secondary infections or asthma exacerbations, although it should be noted that the study was not designed to capture clinical differences in disease severity beyond the need for hospital admission, and we could not differentiate admissions related to influenza from admissions related to other respiratory pathogens. In line with previous research,[12,30] receipt of LAIV-4 tended to be associated with smaller reductions in antibiotics prescription rates, although these results should be interpreted with caution due to the risk of residual confounding and non-specificity of the outcome.

Results from this study only allows inference for a season dominated by influenza A(H3N2), and findings are limited to children aged between 2 and 6 years without previous vaccination for influenza. During the preceding season, influenza circulation was at a historic low due to COVID-19 restrictions, and influenza A(H3N2) had not been circulating widely since the 2018–2019 season, providing opportunity of unique sensitivity to study LAIV-4 effectiveness in children with low or no pre-existing immunity. Immunological studies have shown pre-existing immunity to potentially inhibit immunogenicity and effectiveness of subsequent influenza vaccines.[31] With low or no pre-existing immunity, the vaccine effects detected in the present study would be expected to represent maximal effect sizes, which may not be replicable in settings with higher pre-existing immunity. Similarly, studies have demonstrated reduced vaccine effectiveness after repeated vaccinations when vaccine strains are identical but circulating viruses have drifted.[32,33] Important areas for future research are to understand optimal immunisation ages in children, and how immune responses in children are affected by repeated vaccination with LAIV-4, including whether annual vaccination is the optimal strategy.

Overall, this study adds to the existing literature suggesting that vaccination with LAIV-4 offers moderate effectiveness against influenza-related hospital contacts in children aged between 2 and 6 years, with a vaccine effectiveness of 64·3% during a first-time vaccination schedule, targeting healthy children and a season dominated by influenza A(H3N2). Effectiveness of LAIV-4 was, however, lower against more severe influenza, and LAIV-4 did not provide protection against hospital admissions for respiratory infections, wheezing and asthma, nor against the number of prescriptions of antibiotics. Further multinational and cross-seasonal studies are needed to determine the ability of LAIV-4 to prevent life-threatening illness from influenza, the durability of immune protection from

LAIV-4, and the optimal revaccination schedules. Given the highly variable nature of influenza viruses and suboptimal vaccine effectiveness, even in seasons with good antigenic match between vaccines and circulating strains, we urge the development of influenza vaccines with broad-spectrum protection and durable antibodies.

Contributors

All authors contributed to conceptualisation of the study. HK performed the data analysis, and LCL verified the underlying data and code. HK drafted the original manuscript, and all authors provided critical revision of the article. All authors had full access to study results and have approved the final version for publication.

Declaration of interests

AP reports funds paid to his institution for participation in research projects funded by Alcon, Almirall, Astellas, AstraZeneca, Boehringer Ingelheim, Novo Nordisk, Servier, and LEO Pharma, outside the current work. LCL reports funds paid to his institution for participation in research projects funded by Menarini Pharmaceuticals and LEO Pharma, outside the current work. HK and LGS declare no competing interests.

Data sharing

Due to Danish data protection regulations, individual level data cannot be shared directly by the authors. Data from influenza surveillance in Denmark can be made available for authorised researchers upon application to Statens Serum Institute Denmark. Deidentified data from Danish healthcare registries is accessible for researchers after application to the Danish Health Data Authority. The study protocol was registered in the Real-World Evidence Registry (https://osf.io/havwq) before commencement of statistical analyses.

Acknowledgments

The study was funded by a grant awarded to HK from the philanthropic fund Beckett-Fonden. We thank Peter Bjødstrup Jensen from the University of Southern Denmark for reviewing the source code used in this study.

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