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Estimating vaccine effectiveness against laboratory-confirmed influenza among children and adolescents in Lower Saxony and Saxony-Anhalt, 2012–2016

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SUMMARY

Influenza vaccine effectiveness (VE) has to be estimated anew for every season to explore vaccines' protective effect in the population. We report VE estimates against laboratory-confirmed influenza A(H1N1)pdm09, A(H3N2) and influenza B among children aged 2–17 years, using test-negative design. Pooled data from two German federal states' surveillance systems for acute respiratory illness from week 40/2012 to 20/2016 was used, yielding a total of 10 627 specimens. Odds ratios and 95% confidence intervals (95% CIs) for the association between laboratory-confirmed influenza and vaccination status were calculated by multivariate logistic regression adjusting for age, sex, illness onset and federal state. VE was estimated as 1-Odds Ratio. Overall adjusted VE was 33% (95% CI: 24·3–40·7). A strong variation of VE between the seasons and subtypes was observed: highest season- and subtype-specific VE of 86·2% (95% CI: 41·3–96·7) was found against A(H1N1)pdm09 in 7–17-year-olds in 2015/16. Low estimates of VE were observed against A(H3N2) in any season, e.g. 1·5% (95% CI: −39·3–30·3) in 2014/15. Estimates showed a tendency to higher VE among 7–17-year-old children, but differences were not statistically significant. Although our findings are common in studies estimating influenza VE, we discussed several explanations for observed low VE.

Key words: Epidemiology, influenza (seasonal), routine surveillance data, test-negative design, vaccine effectiveness.

INTRODUCTION

According to estimates for the global burden of seasonal influenza by the World Health Organization (WHO), influenza causes approximately one billion cases and 300 000–500 000 of deaths annually [1].

In Germany, annual vaccination against influenza is recommended for persons older than 60 years. Furthermore, vaccination is recommended by indication for the following risk groups: women in the second or third trimester of pregnancy, people living in nursing homes, persons with high occupational risk (e.g. health personnel) and people suffering from chronic diseases (e.g. asthma, cardiovascular disease, diabetes mellitus, HIV) including children with such conditions [2]. Despite these recommendations, influenza vaccination of healthy children is in most cases also covered by medical insurance companies in Saxony-Anhalt and Lower Saxony.

According to the German vaccination recommendations during the years 2013/14 to 2015/

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16, children aged 2–6 years with an indication for seasonal influenza vaccination should preferably receive live attenuated influenza vaccine (LAIV) instead of inactivated influenza vaccines (IIV). Children with chronic diseases aged 7–17 years could either receive LAIV or IIV [2, 3]. Preferential recommendation of LAIV was cancelled in 2016/17 [4]. Initially, a meta-analysis from Falkenhorst *et al*., comparing the efficacy of LAIV and IIV, showed a higher efficacy of LAIV than of IIV in 2–6-year-old children [5]. However, observational study results from the recent seasons indicated a weak preventive effect of LAIV, especially against influenza A(H1N1)pdm09 [4, 6].

Due to antigenic drift of influenza viruses, vaccine composition has to be adapted annually in order to match circulating viruses and is recommended by the WHO [7–11].

The efficacy of a vaccine is firstly tested under controlled conditions, such as randomised controlled trials (RCT), before a new vaccine gets licensed [12]. For ethical and economic reasons, such trials are not feasible for influenza because influenza vaccine formulation changes annually.

In contrast, vaccine effectiveness (VE) measures the reduction of a disease in the field under ordinary conditions [12]. A commonly used method to estimate the effectiveness of influenza vaccines in the field annually and quickly is the test-negative design (TND) [13–18].

There are several possible factors that could have an impact on influenza VE such as possible changes of the circulating viruses and the composition of the vaccine itself or characteristics of the study population.

Estimating and monitoring VE is important to explore vaccines' protective effect in the target population under ordinary conditions [13]. It may help to recognise a lack of protection and could be helpful when adapting vaccination recommendations and redefining the target population.

This study aims to estimate VE against laboratory-confirmed influenza among 2–17-year-old children and adolescents in two German federal states during four influenza seasons from 2012 to 2016. Therefore, pooled data from both states' virological surveillance systems for acute respiratory illness (ARI) of the Governmental Institute of Public Health in Lower Saxony and the State Agency for Consumer Protection in Saxony-Anhalt was used. We pooled data of these states because of the similar surveillance systems for ARI and a long-term cooperation with the aim of increasing sample size for influenza VE estimation.

METHODS

Study population and study design

We analysed data of 2–17-year-old children from the ARI-sentinel systems of Lower Saxony and Saxony-Anhalt over four consecutive influenza seasons from 2012/13 to 2015/16, each monitoring season lasting from calendar weeks 40 to 20 of the following year.

In Lower-Saxony, 40 general practitioners, predominantly paediatricians and four hospitals participate in the influenza sentinel surveillance system. Samples from nasopharynx of children presenting ARI, defined as pharyngitis, bronchitis or pneumonia with or without fever, are taken from a maximum of seven patients per week by general practitioners and of 15 patients per week by hospitals. Samples are analysed in the laboratory of the Governmental Institute of Public Health of Lower Saxony. The sentinel system from Saxony-Anhalt consists of 15 paediatricians. The procedure of sampling and analysing is similar to the procedure of Lower Saxony and is described in detail elsewhere [19].

The laboratories use real-time reverse transcription polymerase chain reaction (RT–PCR) to test for influenza and other viruses causing acute respiratory symptoms. In addition, practitioners complete a questionnaire, which provides basic demographic data, information on symptoms, on date of illness onset and on vaccination of the respective patient.

The present study comprises 2–17-year-old patients swabbed from nasopharynx. Patients with missing values of vaccination status or vaccinated within 14 days before illness onset were excluded from data analysis as well as patients with missing data on sex or age.

We also excluded data of patients with an onset of illness more than 8 days before the date of sample collection since the viral load, and thus the probability of detecting the virus, decreases over time [20].

If the date of illness onset was missing, it was imputed by 'sampling date minus median-difference between sampling date and illness onset' during the particular season.

We considered a patient as vaccinated when she or he had received at least one dose of any influenza vaccine recommended by the WHO and approved in Germany for the particular season. A case was defined as a patient tested positive for at least one of the influenza virus subtypes A(H1N1)pdm09, A (H3N2) or influenza B. Cases tested positive for

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unsubtyped influenza A were included when estimating VE against any influenza, but excluded for the subtype analyses. When estimating overall influenza VE, a case was counted only once, even if tested positive for more than one subtype (co-infection). In the subtype-analyses, patients with co-infections were considered as a case for every subtype they tested positive for. Controls had to be tested negative for all influenza virus subtypes but could be tested positive for other viruses causing ARI, e.g. picornavirus or adenovirus. Consecutive testing of the same patient could not be identified and was considered as a new participant for every test.

Laboratory methods

Samples of patients with acute onset of respiratory symptoms were analysed qualitatively using RT–PCR to detect RNA of influenza A and B. RT–PCR was conducted in a LightCycler 480 (Roche, Mannheim, Germany) in Lower Saxony and in a Rotor-Gene (Corbett) in Saxony-Anhalt using specific primers and probes covering the matrix gene region of influenza A- and B-genome. A second assay for the subtype-independent detection of influenza A viruses was supplemented with a set of PCR systems that allowed differentiation of haemagglutinin and neuraminidase subtypes including influenza A(H1N1) pdm09 and A(H3N2) viruses. Positive samples were sent to the National Reference Centre for Influenza at the Robert Koch-Institute (Berlin, Germany) for sequence analyses and nationwide influenza surveillance.

Statistical analysis

We used TND for estimation of influenza VE. We estimated the association of influenza virus confirmation with vaccination status via Odds ratios (OR) calculated by using multivariate logistic regression adjusting for age, sex, month of illness onset and federal state. For estimating VE against laboratory-confirmed influenza, we used the formula 1-OR. VE was considered statistically significant when 95% confidence limits (95% CIs) excluded zero.

Separate analyses were conducted for influenza virus subtypes (A(H1N1)pdm09, A(H3N2) and influenza virus B) and for any influenza virus. The analyses were stratified for two age groups (2–6 years and 7–17 years). VE was reported if 30 or more cases of influenza occurred.

We did not conduct separate analyses for types of vaccine (LAIV *vs.* IIV) since information about the used type of vaccine has been collected for Lower Saxony only since 2016.

In order to characterise influenza seasons, season-specific influenza positive proportions were calculated as the number of patients tested positive for influenza divided by the number of all tested patients. We conducted sensitivity analysis for VE in 2015/16, excluding children with missing date of illness onset instead of imputing the date.

Statistical analyses were conducted using IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp. Released 2015. Armonk, NY).

Ethical aspects

Samples and questionnaires were collected for the purpose of routine virological sentinel surveillance by the State Health Agencies of Lower Saxony and Saxony-Anhalt to monitor the course and severity of ARI caused by different viruses, including influenza virus. For the analysis of VE, data were completely anonymised so that patients could not be identified. Since VE analysis was a secondary data analysis based on routine surveillance data, ethical approval was not regarded as necessary [21].

RESULTS

Description of the study population and influenza seasons

Between 28 September 2012 and 28 May 2016, 11 316 samples of children aged 2–17 years were analysed within the monitoring systems of Lower Saxony and Saxony-Anhalt.

We excluded 689 specimens that did not meet the inclusion criteria (e.g. missing information on vaccination status or sex) which yielded a final dataset of 10 627 specimens with 7333 (69·0%) samples from Lower Saxony and 3294 (31·0%) from Saxony-Anhalt (Table 1).

A total of 5644 (53·1%) patients were male and the overall median age was 5·6 years (mean age 7·0 years). Of all patients, 6245 (58·8%) were aged 2–6 years and 4382 (41·2%) belonged to age group 7–17 years (Table 1). Across all seasons, 7·5% of all patients and 8·1% of the controls were vaccinated. The latter group can be regarded as an estimate for the population seeking health care. In this group, the seasonal

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Table 1. *Characteristics of the study population*

Variable	Cases (%), $n = 3304$	Controls (%), $n = 7323$	Total (%), $N = 10\,627$
Federal state			
Lower Saxony	2319 (70·2)	5014 (68·5)	7333 (69·0)
Saxony-Anhalt	985 (29·8)	2309 (31·5)	3294 (31·0)
Sex			
Male	1788 (54·1)	3856 (52·7)	5644 (53·1)
Female	1516 (45·9)	3467 (47·3)	4983 (46·9)
Age group			
2–6	1725 (52·2)	4520 (61·7)	6245 (58·8)
7–17	1579 (47·8)	2803 (38·3)	4382 (41·2)
Vaccinated			
Yes	209 (6·3)	591 (8·1)	800 (7·5)
No	3095 (93·7)	6732 (91·9)	9827 (92·5)
Disease onset			
Sept	0 (0·0)	26 (0·4)	26 (0·2)
Oct	3 (0·1)	720 (9·8)	723 (6·8)
Nov	40 (1·2)	874 (11·9)	914 (8·6)
Dec	116 (3·5)	761 (10·4)	877 (8·3)
Jan	629 (19·0)	1330 (18·2)	1959 (18·4)
Feb	1323 (40·0)	1492 (20·4)	2815 (26·5)
Mar	947 (28·7)	1331 (18·2)	2278 (21·4)
Apr	231 (7·0)	620 (8·5)	851 (8·0)
May	15 (0·5)	169 (2·3)	184 (1·7)

Table 2. *Vaccination coverage, 2012–2016*

	Vaccination coverage*					
Season	Total	Cases Vaccin. (%)*	Total	Controls Vaccin. (%)	Total	Total Vaccin. (%)
2012/13	1443	67 (4·6)	1792	146 (8·1)	3235	213 (6·6)
2–6-year-olds	724	33 (4·6)	1149	76 (6·6)	1873	109 (5·8)
7–17-year-olds	719	34 (4·7)	643	70 (10·9)	1362	104 (7·6)
2013/14	152	16 (10·5)	1867	144 (7·7)	2019	160 (7·9)
2–6-year-olds	95	9 (9·5)	1174	75 (6·4)	1269	84 (6·6)
7–17-year-olds	57	7 (12·3)	693	69 (10·0)	750	76 (10·1)
2014/15	940	82 (8·7)	2071	179 (8·6)	3011	261 (8·7)
2–6-year-olds	495	34 (6·9)	1225	93 (7·6)	1720	127 (7·4)
7–17-year-olds	445	48 (10·8)	846	86 (10·2)	1291	134 (10·4)
2015/16	769	44 (5·7)	1593	122 (7·7)	2362	166 (7·0)
2–6-year-olds	411	22 (5·4)	972	63 (6·5)	1383	85 (6·1)
7–17-year-olds	358	22 (6·1)	621	59 (9·5)	979	81 (8·3)
2012–2016	3304	209 (6·3)	7323	591 (8·1)	10 627	800 (7·5)
2–6-year-olds	1725	98 (5·7)	4520	307 (6·8)	6245	405 (6·5)
7–17-year-olds	1579	111 (7·0)	2803	284 (10·1)	4382	395 (9·0)

* Number (percentage) of vaccinated patients.

coverages varied slightly between 7·7% and 8·6%, not showing a substantial trend. Vaccination coverage was slightly higher in 7–17-year-olds than in the younger ones. Season-, age group- and group-specific vaccination coverage is shown in Table 2.

The majority of samples (7052 of 10 627, 66·4%) was taken between January and March.

In total, 3304 (31·1%) patients tested positive for influenza. Cases were slightly older than controls (median age 6·5 years *vs.* 5·5 years). Among all

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Table 3. *Estimated vaccine effectiveness (VE) against laboratory-confirmed influenza (all subtypes) and influenza A(H1N1)pdm09 stratified for age groups, 2012–2016*

		Any influenza				A(H1N1)pdm09			
Season	Age group	Cases (vaccin.)	Controls (vaccin.)	Crude VE in % (95% CI)	Adjusted* VE in % (95% CI)	Cases (vaccin.)	Controls (vaccin.)	Crude VE in % (95% CI)	Adjusted* VE in % (95% CI)
2012/13	2–17	1443 (67)	1792 (146)	**45·1 (26·1–59·2)**	**50·7 (32·5–64·0)**	278 (11)	1792 (146)	**53·6 (13·1–75·2)**	**56·7 (17·1–77·4)**
	2–6	724 (33)	1149 (76)	32·6 (−2·6–55·7)	**37·1 (2·2–59·6)**	164 (7)	1149 (76)	37·1 (−39·0–71·5)	33·1 (−52·8–70·7)
	7–17	719 (34)	643 (70)	**59·4 (37·9–73·4)**	**57·8 (33·5–73·2)**	114 (4)	643 (70)	**70·2 (16·8–89·4)**	**73·2 (22·7–90·7)**
2013/14	2–17	152 (16)	1867 (144)	−40·8 (−142·8–18·4)	−4·3 (−2·9–40·5)	27 (1)	1867 (144)	†	†
	2–6	95 (9)	1174 (75)	−53·3 (−216·8–25·8)	−25·5 (−165·5–40·7)	18 (1)	1174 (75)	†	†
	7–17	57 (7)	693 (69)	−26·6 (−190·1–44·7)	8·8 (−114·0–61·2)	9 (0)	693 (69)	†	†
2014/15	2–17	940 (82)	2071 (179)	−1·0 (−32·8–23·2)	22·3 (−4·7–42·3)	182 (15)	2071 (179)	5·1 (−64·6–45·2)	9·3 (−62·0–49·3)
	2–6	495 (34)	1225 (93)	10·2 (−34·9–40·3)	22·7 (−21·8–50·9)	133 (9)	1225 (93)	11·7 (−79·5–56·5)	21·3 (−67·7–63·0)
	7–17	445 (48)	846 (86)	−6·8 (−55·2–26·4)	21·6 (−17·0–47·5)	49 (6)	846 (86)	−23·3 (−198·1–49·0)	−8·0 (−173·0–57·3)
2015/16	2–17	769 (44)	1593 (122)	26·8 (−4·5–48·7)	**49·4 (25·7–65·5)**	324 (15)	1593 (122)	41·5 (−1·5–66·2)	**60·2 (29·3–77·6)**
	2–6	411 (22)	972 (63)	18·4 (−34·5–50·5)	**44·8 (4·6–68·0)**	219 (13)	972 (63)	8·9 (−68·6–50·8)	44·8 (−6·9–71·5)
	7–17	358 (22)	621 (59)	37·6 (−3·6–62·5)	**52·9 (18·4–72·8)**	105 (2)	621 (59)	**81·5 (23·1–95·6)**	**86·2 (41·3–96·7)**
2012–2016‡	2–17	3304 (209)	7323 (591)	**23·1 (9·4–34·7)**	**33·0 (24·3–40·7)**	811 (42)	7323 (591)	**37·8 (14·2–54·9)**	**47·0 (25·7–62·2)**
	2–6	1725 (98)	4520 (307)	17·3 (−4·5–34·6)	**27·8 (6·8–44·1)**	534 (30)	4520 (307)	18·3 (−20·2–44·5)	**34·6 (1·0–56·8)**
	7–17	1579 (111)	2803 (284)	**32·9 (15·7–46·6)**	**40·1 (23·0–53·4)**	277 (12)	2803 (284)	**59·8 (27·4–77·8)**	**63·6 (33·1–80·3)**

Statisically significant estimates (95% confidence limits excluding zero) are printed in bold.

* Adjusted for sex, age, month of illness onset and federal state.

† No VE estimate because of a too small number of cases (<30).

‡ Adjusted estimates additionally adjusted for season.

Table 4. *Estimated vaccine effectiveness (VE) against laboratory-confirmed influenza A(H3N2) and influenza B stratified for age groups, 2012–2016*

		A(H3N2)				B			
Season	Age group	Cases (vaccin.)	Controls (vaccin.)	Crude VE in % (95% CI)	Adjusted* VE in % (95% CI)	Cases (vaccin.)	Controls (vaccin.)	Crude VE in % (95% CI)	Adjusted* VE in % (95% CI)
2012/13	2–17	472 (30)	1792 (146)	23·5 (−14·9–49·0)	30·2 (−7·1–54·5)	696 (26)	1792 (146)	**56·3 (33·0–71·4)**	**58·6 (34·0–74·0)**
	2–6	307 (15)	1149 (76)	27·5 (−28·1–58·9)	29·7 (−27·9–61·4)	256 (11)	1149 (76)	36·6 (−21·1–66·8)	41·1 (−18·5–70·7)
	7–17	165 (15)	643 (70)	18·1 (−47·1–54·4)	23·3 (−43·6–59·1)	440 (15)	643 (70)	**71·1 (48·8–83·7)**	**65·8 (35·8–81·8)**
2013/14	2–17	107 (13)	1867 (144)	−65·5 (−202·8–9·6)	−18·6 (−120·8–36·3)	14 (2)	1867 (144)	†	†
	2–6	70 (7)	1174 (75)	−62·8 (−267·9–27·9)	−22·5 (−182·9–46·9)	4 (1)	1174 (75)	†	†
	7–17	37 (6)	693 (69)	−75·0 (−334·3–29·5)	−17·6 (−201·0–54·0)	10 (1)	693 (69)	†	†
2014/15	2–17	527 (55)	2071 (179)	−23·2 (−69·4–10·4)	1·5 (−39·2–30·3)	210 (11)	2071 (179)	41·6 (−9·3–68·8)	**60·3 (23·5–79·4)**
	2–6	283 (22)	1225 (93)	−2·6 (−66·4–36·8)	9·3 (−54·3–46·7)	69 (3)	1225 (93)	44·7 (−79·4–82·9)	54·9 (−51·7–86·6)
	7–17	244 (33)	846 (86)	−38·2 (−112·3–10·0)	−5·6 (−68·3–33·7)	141 (8)	846 (86)	46·8 (−12·3–74·8)	**63·6 (20·3–83·4)**
2015/16	2–17	10 (1)	1593 (122)	†	†	423 (28)	1593 (122)	14·5 (−30·8–44·1)	**42·3 (7·2–64·2)**
	2–6	8 (1)	972 (63)	†	†	176 (8)	972 (63)	31·3 (−46·0–67·7)	47·1 (−22·5–77·2)
	7–17	2 (0)	621 (59)	†	†	247 (20)	621 (59)	16·1 (−42·6–50·6)	35·6 (−15·9–64·2)
2012–2016‡	2–17	1116 (99)	7323 (591)	−10·9 (−38·6–11·3)	8·4 (−16·6–28·0)	1343 (67)	7323 (591)	**40·2 (22·5–53·9)**	**50·7 (34·3–63·0)**
	2–6	668 (45)	4520 (307)	0·9 (−37·0–28·3)	13·0 (−23·2–38·6)	505 (23)	4520 (307)	34·5 (−1·1–57·6)	**40·9 (5·1–63·2)**
	7–17	448 (54)	2803 (284)	−21·6 (−65·7–10·8)	2·6 (−36·8–30·7)	838 (44)	2803 (284)	**50·8 (31·8–64·6)**	**53·8 (33·5–67·9)**

Statisically significant estimates (95% confidence limits excluding zero) are printed in bold.

* Adjusted for sex, age, month of illness onset and federal state.

† No VE estimate because of a too small number of cases (<30).

‡ Adjusted estimates additionally adjusted for season.

patients tested positive, 811 (24·5%) tested positive for A(H1N1)pdm09, 1116 (33·8%) for A(H3N2), 46 for unsubtyped influenza A (1·4%) and 1343 (40·6%) for influenza B. Twelve patients had co-infections with two different subtypes of influenza.

Influenza-positive proportions during the seasons 2012/13, 2014/15 and 2015/16 were higher than 30% (44·6%, 1443 cases; 31·2%, 940; 32·6%, 769), whereas the positive proportion in 2013/14 was only 7·5% (152).

The proportion of A(H1N1)pdm09 of all cases was highest in 2015/16 with 42·1% (324 cases) and below 20% during the other seasons. Influenza A(H3N2) was the predominant subtype in 2013/14 (107 cases, 70·4%) and 2014/15 (527, 56·1%). Influenza B was predominant in 2012/13 (696, 48·2%) and 2015/16 (423, 55·0%).

Vaccine effectiveness

Any influenza

Crude estimate for VE against any influenza across all seasons was 23·1% (95% CI: 9·4–34·7), adjusted estimate for VE against any influenza across all seasons was 33·0% (95% CI: 24·3–40·7) for all age groups (Table 3). Season-specific VE estimates indicated a preventive effect being statistically significant only for seasons 2012/13 (50·7%, 95% CI: 32·5–64·0) and 2015/16 (49·4%, 95% CI: 25·7–65·5). Point estimates of VE were higher for age group 7–17 years than for the younger ones, but the differences were not statistically significant. This was also the case in the following subtype-analyses (Table 3).

A(H1N1)pdm09

Across all seasons, adjusted estimate for VE against laboratory-confirmed A(H1N1)pdm09 was 47·0% (95% CI: 25·7–62·2) among both age groups. VE estimates across both age groups were 56·7% (95% CI: 17·1–77·4) in 2012/13 and 60·2% (95% CI: 29·3–77·6) in 2015/16. Whereas estimated VE for 7–17-year-olds was 73·2% (95% CI: 22·7–90·7) in 2012/13 and 86·2% (95% CI: 41·3–96·7) in 2015/16, estimates for children aged 2–6 years were 33·1% (95% CI: −52·8–70·7) and 44·8% (95% CI: −6·9–71·5), respectively. Estimated VE was 9·3% (95% CI: −62·0–49·3) in 2014/15. A negative point estimate was obtained for children aged 7–17 years in 2014/15 (−8·0, 95% CI: −173·0–57·3) (Table 3).

A(H3N2)

Estimated VE against A(H3N2) across all seasons was 8·4% (95% CI: −16·6–28·0). Highest estimate was 30·2% (95% CI: −7·1–54·5) in season 2012/13. We obtained negative estimates in 2013/14 (−18·6%, 95% CI: −120·8–36·3) among both age groups. In 2014/15, estimated VE was 1·5% (95% CI: −39·2–30·3), 9·3% (95% CI: −54·3–46·7) for children aged 2–6 years and −5·6% (95% CI: −68·3–33·7) for 7–17-year-old children (Table 4).

Influenza B

Adjusted VE estimate against laboratory-confirmed influenza B among all children across all seasons was 50·7% (95% CI: 34·3–63·0). Highest VE estimates across both age groups were 58·6% (95% CI: 34·0–74·0) in 2012/13 and 60·3% (95% CI: 23·5–79·4) in 2014/15. In 2012/13, estimated VE was 65·8% (95% CI: 35·8–81·8) for 7–17-year-old children and 41·1% (95% CI: −18·5–70·7) for 2- to 6-year-olds. Same patterns were observed in 2014/15. VE was estimated to be 42·3% (95% CI: 7·2–64·2) in 2015/16 (Table 4).

Sensitivity analysis

Date of illness onset was missing for 316 (13·4%) children. Excluding data of patients with missing date of illness onset, VE against any influenza in 2015/16 was 46·4% (95% CI: 17·7–65·1) across both age groups. We observed VE of 39·9% (95% CI: −10·2–67·3) in 2- to 6-year-olds and 50·3% (95% CI: 8·0–73·1) in children aged 7 to 17 years.

DISCUSSION

We estimated VE against laboratory-confirmed influenza for four influenza seasons among children and adolescents using TND and routine surveillance data from Lower Saxony and Saxony-Anhalt.

We found low to moderate values of VE with an average of 33% across all seasons. A moderate preventive effect of about 50% was found for seasons 2012/13 and 2015/16. A strong variation of VE between the seasons and subtypes was observed. VE against A(H1N1)pdm09 ranged from −8% (95% CI: −173·0–57·3) for 7–17-year-olds in 2014/15 to 86·2% (95% CI: 41·3–96·7) for 7–17-year-olds in 2015/16. In general, there was a moderate VE against this subtype in 2012/13 and in 2015/16, whereas VE against A(H3N2) was low for every season, ranging from

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−22·5% (95% CI: −182·9–46·9) for 2–6-year-olds in 2013/14 to a maximum of 30·2% (95% CI: −7·1–54·5) across both age groups in 2012/13. Against influenza B, highest values were observed for 7–17-year-old children in 2012/13 (65·8%, 95% CI: 35·8–81·8) and 2014/15 (63·6%, 95% CI: 20·3–83·4). Low values were observed in 2015/16, when the lowest estimate of 35·6% (95% CI: −15·9–64·2) was obtained for 7–17-year-olds. Estimated values for VE are similar compared to the results of other VE studies [13–19].

We conducted sensitivity analysis for 2015/16, excluding 316 children with missing date of illness onset instead of imputing the date. VE of sensitivity analysis did not differ relevantly from main results (46·4%, 95% CI: 17·7–65·1 *vs.* 49·4%, 95% CI: 25·7–65·5 in 2- to 17-year-olds).

Observed low VE against A(H3N2), especially in 2014/15, could be explained by a mismatch between circulating strains and vaccine strains. There was a good match for A(H1N1)pdm09 through the seasons what coincides with observed moderate VE in 2012/13 and 2015/16. Matches were found for influenza B except from 2015/16, when trivalent vaccine did not include a strain of the circulating line [8–11, 22–25].

We found a tendency to higher estimates of VE in children aged 7–17 years for some subtypes and seasons. One possible explanation might be the type of vaccine (LAIV or IIV) itself. Although studies from Finland and the UK showed a preventive effect of LAIV [17, 19, 26, 27], a relatively low or even a lack of preventive effect of LAIV compared with IIV during 2013–2016 was found elsewhere [28]. CDC's Advisory Committee on Immunization Practices (ACIP) therefore voted against the use of LAIV in 2016/17 [28]. We could not stratify directly for vaccine type. If paediatricians complied with the national recommendation, the 2–6-year-olds in our study might have received LAIV more often than older children from 2013/14 to 2015/16. This could be one out of several possible explanations for low VE estimates among this age group but should be interpreted with caution as no coverage data on vaccine type was available. Helmeke *et al.* examined VE by vaccine type using pooled data from Lower Saxony and Saxony-Anhalt from season 2015/16 and found a lower VE of LAIV, especially against A(H1N1)pdm09, compared with IIV in children [29].

We observed some, statistically not significant, negative point estimates of VE, e.g. −5·6% (95% CI: −68·3–33·7) against A(H3N2) for 7–17-year-old children in 2014/15. Due to the formula VE = 1-OR, the estimate was between 0% and 100% if cases had a lower odds for being vaccinated than controls, and below zero per cent if this was not the case. Even estimates below −100% were possible. Besides random variation, there are further hypotheses explaining the occurrence of negative VE, namely the antigenic distance hypothesis and repeat vaccination effects, as described elsewhere [30, 31]. Skowronski *et al.* found low VE against A(H3N2) in Canada in 2014/15 and mentioned repeated vaccination effects, variation of virus' genome and a mismatch between wild strain and vaccine strain as possible explanation [32, 33].

Nevertheless, there might be some biases and methodological issues that have to be addressed.

Firstly, information bias, particularly misclassification error, leading to underestimation of VE, has to be taken into account. Misclassification of cases and controls and associated underestimation of VE is negligible in TND when using high sensitive and specific test methods [34, 35]. Hence, this kind of bias can be neglected in our study, as we used highly sensitive and specific RT–PCR for diagnosis of laboratory-confirmed influenza.

Relating to the exposure collected by the questionnaire, particularly vaccination status and date, this information could have been incorrect, as patients might not have remembered vaccination correctly. When assessing this kind of misclassification error, it has to be considered that in Germany, though there is free choice of physician, people usually attend only one and the same general physician or paediatrician. If the paediatrician who had provided the vaccine before was the same paediatrician who took the sample, he or she should have reported vaccination status correctly.

Secondly, the occurrence of selection bias has to be taken into account. In Germany, the Standing Committee on Vaccination does not recommend influenza vaccination for children in general, but for people with underlying chronic diseases [2]. We expect that children with underlying chronic conditions were overrepresented in our study because they were more likely to consult a paediatrician and thus to be sampled due to their chronic diseases. Nevertheless, there are no hints for a poorer VE in people with underlying chronic disease. That is why we do not think that this substantially affects our results.

Additionally, another kind of selection bias has to be considered. In principal, the paediatricians were asked to select the patients randomly and not according to further criteria besides the clinical case definition of ARI. However, it cannot be excluded that

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vaccinationstatus of the patient or the severity of the disease might have influenced the indication for swabbing in some cases. However, the proportion of vaccinated children was quite low in our sample and very severe cases would rather have attended hospital, not the paediatrician. Thus, as hospital patients represent only a minority of our sample, it is unlikely that this kind of bias has serious impact on our results.

We conclude that there might be several biases that can influence the VE estimates and thus might explain the low estimates, but none of these biases should have substantial effects on our results. However, for the future it is planned to gain more information about underlying chronic diseases, to better address selection bias.

As the study is based on secondary data, we could not obtain additional data on further details of vaccination or confounders. For instance, we had no information on vaccine type, number of received doses and whether the children had received the vaccine for the first time. We added these aspects to our surveillance questionnaire so that we will have this information for future analyses. The main confounders considered in this analysis were month of illness onset, age, sex and season. Not surprisingly, the season had an effect on VE estimates, as the predominating virus subtypes and the vaccine composition differed between the seasons, leading to a better or poorer match between circulating viruses and vaccine viruses. Month of illness onset had an impact on VE estimates as well. We chose February as reference as this month is the peak time of influenza seasons. The risk of an infection with influenza viruses decreased with increasing distance of time from February. Impact of age, federal state and sex varied between the seasons and subtypes.

We accounted for random variation by providing CIs for VE estimates. Many season-specific CIs included an effect of zero per cent. CIs were quite wide because the vaccination coverage of children in the study population was below 10%. Over the study period, there were no changes of the vaccination recommendations for children and thus the vaccination coverage in our study population did not change substantially either. The higher coverage in the older children might be due to the fact that some chronic diseases manifest with increasing age. It would also be conceivable, that parents' experience with influenza and influenza-like illness in their children changes over time and that this aspect plays a role when deciding to get the children vaccinated. The low coverage made it difficult to gain sufficiently large sample sizes for statistically significant estimates. However, compared with similar studies, our sample sizes per season were rather high, especially because we pooled data from two federal states. The primary purpose of the surveillance systems does not justify to further increase sample size. Thus, if higher precision and smaller CIs are required, results from several studies should be brought together by means of meta-analysis (see e.g. Belongia *et al.*, 2016 [18]).

A significant preventive effect of the vaccine against any influenza was found for two out of four observed influenza seasons (2012/13 and 2015/16). A lack of preventive effect, low or even moderate VE estimates, e.g. 49·4% against any influenza in 2015/16, could be problematic when communicating benefits of influenza vaccination to the population. Such values might not help increase vaccination uptake or encourage personal decisions of getting vaccinated. Therefore, communication of VE estimates requires caution. A VE of 50% should not be confused with 50 out of 100 vaccinated people getting infected with influenza anyway. It needs to be communicated that it still means that the odds of getting ill for the vaccinated ones' is half of the odds of people who did not receive the vaccine. Even if a better evidence of VE might encourage more people to get vaccinated, in long-term, it might be helpful to communicate that influenza vaccine does not have 100% effectiveness.

Although RCT is the best design to measure vaccine's efficacy [12], observational studies are needed to measure influenza VE in the field. For this purpose, TND is a commonly used method and produces acceptable estimates for true VE [34, 36].

In conclusion, we found a strong variation of vaccines' preventive effect against laboratory-confirmed influenza between the observed seasons and the different subtypes. High values of VE were found against A (H1N1)pdm09, especially in 7–17-year-olds, e.g. 86·2% (95% CI: 41·3–96·7) in 2015/16 and 73·2% (95% CI: 22·7–90·7) in 2012/13. Low estimates were observed against A(H3N2) in any season, e.g. 1·5% (95% CI: −39·3–30·3) in 2014/15 and against A (H1N1)pdm09 in 2014/15 when observed VE across both age groups was 9·3% (95% CI: −62·0–49·3). With regard to the different circulation of influenza virus subtypes during different seasons, low numbers of cases and mentioned explanations for possible underestimation of VE in TND have to be considered here.

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DECLARATION OF INTEREST

None.

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