

# Age-related prevalence of cross-reactive antibodies against influenza A(H3N2) variant virus, Germany, 2003 to 2010

**B Blümel (benjamin.blumel@gmail.com)<sup>1,2,3,4</sup>, B Schweiger<sup>1</sup>, M Dehnert<sup>1,5</sup>, S Buda<sup>1</sup>, A Reuss<sup>1</sup>, I Czogiel<sup>1</sup>, P Kamtsiuris<sup>1</sup>, M Schlaud<sup>1</sup>, C Poethko-Müller<sup>1</sup>, M Thamm<sup>1</sup>, W Haas<sup>1</sup>**

1. Robert Koch Institute, Berlin, Germany

2. Postgraduate Training for Applied Epidemiology (PAE, German FETP), Robert Koch-Institute, Berlin, Germany

3. European Programme for Intervention Epidemiology Training (EPIET), European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden

4. Current affiliation: Institute of Medical Microbiology and Hygiene, University Medical Center Freiburg, Freiburg, Germany

5. Current affiliation: Department of Biotechnology and Bioinformatics, Weihenstephan-Triesdorf University of Applied Sciences, Freising, Germany

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To estimate susceptibility to the swine-origin influenza A(H3N2) variant virus (A(H3N2)v) in the German population, we investigated cross-reactive antibodies against this virus and factors associated with seroprotective titre using sera from representative health examination surveys of children and adolescents (n=815, 2003–06) and adults (n=600, 2008–10). Antibodies were assessed by haemagglutination inhibition assay (HI); in our study an HI titre  $\geq 40$  was defined as seroprotective. We investigated associated factors by multivariable logistic regression. Overall, 41% (95% confidence interval (CI): 37–45) of children and adolescents and 39% (95% CI: 34–44) of adults had seroprotective titres. The proportion of people with seroprotective titre was lowest among children younger than 10 years (15%; 95% CI: 7–30) and highest among adults aged 18 to 29 years (59%; 95% CI: 49–67). Prior influenza vaccination was associated with higher odds of having seroprotective titre (odds ratio (OR) for children and adolescents: 3.4; 95% CI: 1.8–6.5; OR for adults: 2.4; 95% CI: 1.7–3.4). Young children showed the highest and young adults the lowest susceptibility to the A(H3N2)v virus. Our results suggest that initial exposure to circulating seasonal influenza viruses may predict long-term cross-reactivity that may be enhanced by seasonal influenza vaccination.

## Introduction

Pigs can serve as hosts for the reassortment of influenza viruses of different origins (avian, human and swine) which involves the risk of emergence of new, genetically different influenza viruses that may infect humans. In addition, influenza viruses in pigs do not develop as much genetic diversity in a given time

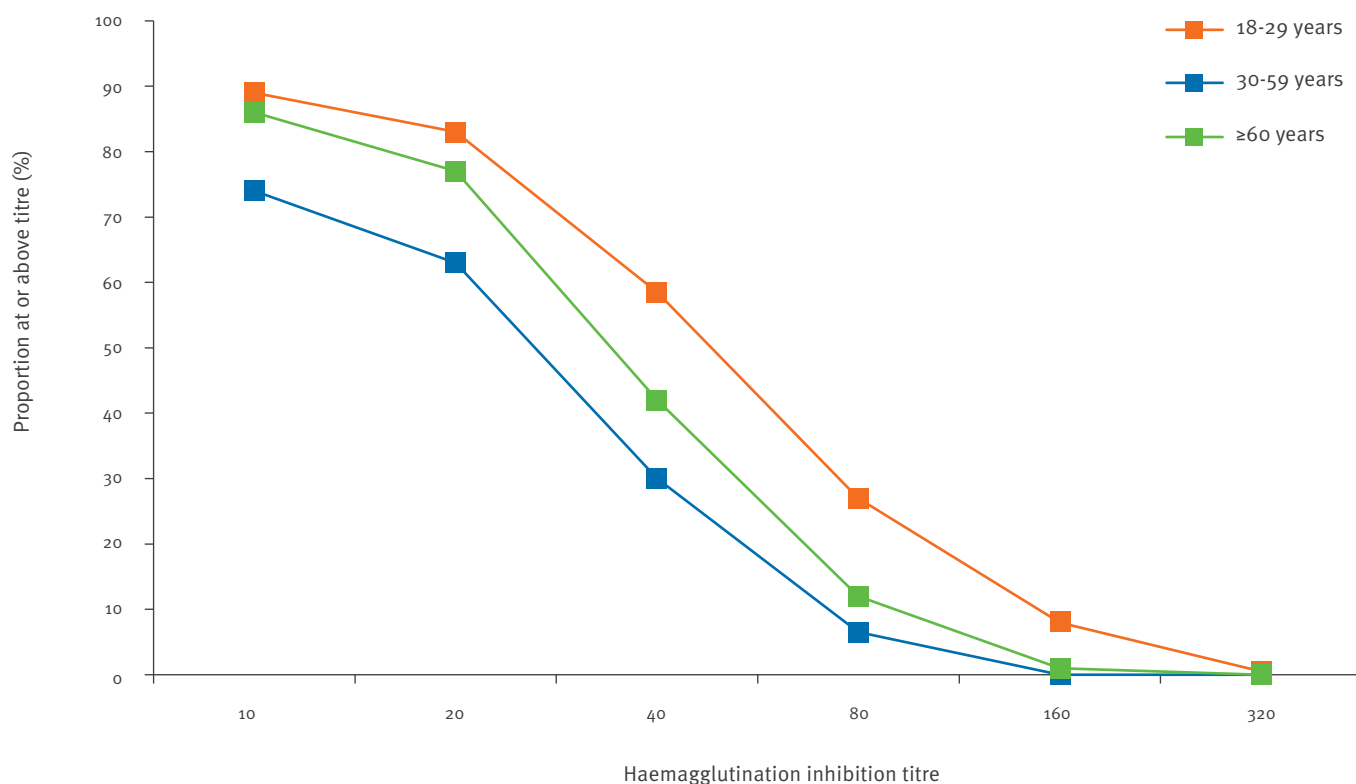
period as those in humans, resulting over time in divergent antigenic characteristics between viruses circulating in swine and humans that may make humans more susceptible to swine viruses [1]. When such zoonotic swine influenza viruses are isolated from humans, they are called variant viruses [2]. The emergence of the pandemic influenza A(H1N1)pdm09 virus in 2009 confirmed the risk posed by zoonotic influenza viruses emerging from pigs.

More than 300 infections with a variant influenza A(H3N2) virus (A(H3N2)v) have been recorded in the United States (US) since 2011, and most cases occurred in 2012 [3]. Most influenza A(H3N2)v infections occurred outside of the influenza season in children, and the course and severity of illness were similar to that of seasonal influenza. Most of the infected persons reported to have attended agricultural fairs where they had either direct or indirect contact with pigs. Limited human-to-human transmission of the A(H3N2)v virus may have occurred as some cases in 2011 did not have any recent history of contact with pigs [4].

The A(H3N2)v virus is a triple reassortant A(H3N2) virus that acquired the matrix protein gene of the A(H1N1)pdm09 virus [5,6]. It is closely related to human A(H3N2) virus strains, represented by the vaccine reference strain A/Wuhan/359/95, that circulated among humans in the mid-1990s until 1997 [5]. The observed age-related pattern of influenza A(H3N2)v infections in the US is most likely due to the similarity of the haemagglutinin of the A(H3N2)v virus to that of A/Wuhan/359/95-like viruses as most cases were born after 1997 and not exposed to such human A(H3N2) viruses. The acquisition of the matrix gene after the

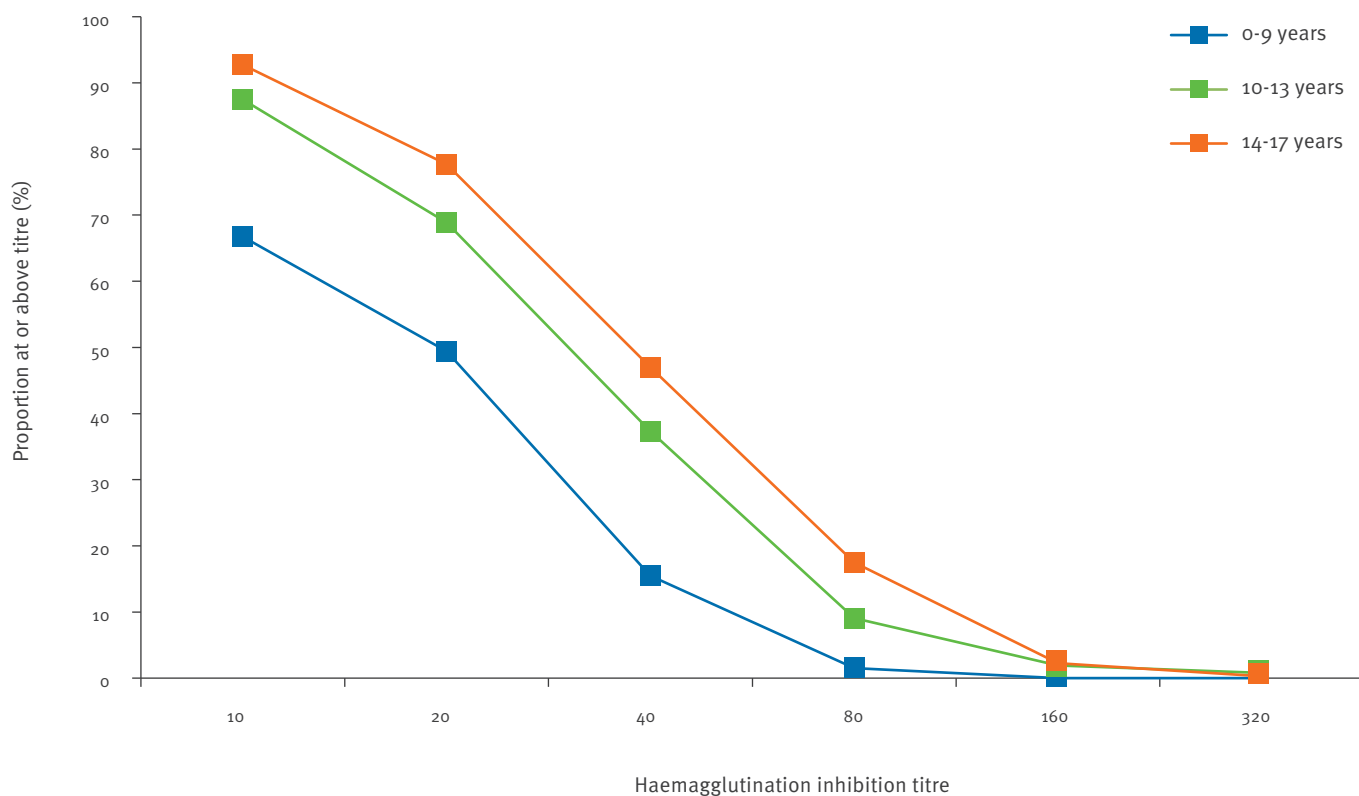
**FIGURE 1**

Reverse cumulative distribution curve of haemagglutination inhibition antibody titres against influenza A/Wisconsin/12/2010, by age group, adults, Germany, 2008–2010 (n = 600)



**FIGURE 2**

Reverse cumulative distribution curve of haemagglutination inhibition antibody titres against influenza A/Wisconsin/12/2010, by age group, children and adolescents, Germany, 2003–2006 (n = 815)



Proportions are weighted (see Methods).

pandemic in 2009, possibly resulting in increased swine–human transmissibility of the virus, may explain the increasing number of A(H3N2)v infections. The low level of cross-reactive antibodies observed among young children and some adult age groups in seroepidemiologic studies in Canada, Japan, Norway, the United Kingdom (UK), and the US, may pose a risk of pandemic emergence of the A(H3N2)v virus [7–11]. A vaccine virus has been developed in case an influenza A(H3N2)v virus vaccine needs to be produced [7,12].

No data are available on the prevalence of cross-reactive antibodies against the A(H3N2)v virus in the population of Germany and most of Western Europe. While infections with the A(H3N2)v virus have not been reported in Europe to date, this cannot be excluded in the future, and further adaptation of the virus to humans, involving increased ability of human-to-human spread, is possible. In a seroepidemiological study among adults, we showed that age-related seroprevalence patterns of antibodies against the A(H1N1) pdm09 virus in Germany did not follow those observed in other countries in Europe and North America [13]. Such variability may, among other reasons, be due to the circulation of different seasonal influenza virus subtypes in the different countries or to different vaccination histories in the studied populations.

To estimate the susceptibility to influenza A(H3N2)v in the German population, we assessed the prevalence of cross-reactive antibodies against this virus as a correlate of pre-existing protection and investigated the association between characteristics of the study population and the prevalence of cross-reactive antibodies.

## Methods

### Serum samples

The serum samples used for this study originated from two population-wide, representative health examination surveys conducted in Germany. For the adult population we used sera from the first wave of the German Health Interview and Examination Survey for Adults (DEGS), conducted from November 2008 through December 2011 [13,14]. DEGS is part of the national health monitoring and combines periodic health surveys with a longitudinal study of adults 18 years or older. Participants (8,152 persons) were randomly selected from the general population by using a stratified two-stage cluster sample approach. The study included completion of standardised questionnaires, physical examinations and tests, and a blood sample (available for 7,116 of the 8,152 participants). For our adult study population, we used an age-stratified (three age groups) random sub-sample of DEGS sera (n=600, 200 in each age group) collected from November 2008 through April 2010 [13].

To assess the seroprevalence in children and adolescents we used sera from the nationwide, population-based German Health Interview and Examination

Survey for Children and Adolescents (KiGGS) that assessed persons between 0 and 17 years of age. Data and blood samples (1–17 years of age) were collected from 2003 through 2006 using an approach similar to DEGS [15]. From the 17,641 participants in the original study, a simple random sub-sample of those for whom blood samples were available (n=815 of 14,387 participants) was used for our study. The reason why the sub-sample for children and adolescents was larger than the adult sample was that these sera were also used in a study of another research group and were thawed and aliquoted at the same time; this allowed us to increase our power for the analysis.

### Serological analysis

We performed haemagglutination inhibition (HI) testing to analyse sera for cross-reactive antibodies against three influenza A(H3N2) virus strains. Influenza A/Wisconsin/12/2010 (A/Wisconsin) was used as representative for the A(H3N2)v virus, the primary target of the study. To confirm the specificity of measured antibody titres against A/Wisconsin, we also tested sera for antibodies against two other A(H3N2) virus strains. One of them, A/Niedersachsen/59/2007 (A/Niedersachsen), was a swine A(H3N2) virus circulating among pigs in Germany that caused one human infection in Germany in 2009 [16] and is closely related to the A(H3N2) swine viruses that emerged in Europe around 1980 after reassortment between human A(H3N2) viruses and swine viruses of subtype A(H1N1). The second strain used for comparison, A/Perth/16/2009 (A/Perth), was the A(H3N2) vaccine strain used in the influenza seasons 2010/11 and 2011/12, representing human influenza A(H3N2) viruses circulating recently [16]. The reference strains A/Wisconsin/12/2010 and A/Perth/16/2009 were obtained from the World Health Organization (WHO) Collaborating Centre in London, UK. A/Niedersachsen was isolated in Lower Saxony and belongs to the influenza virus strain selection of the German National Influenza Reference Centre.

The HI tests were performed according to standard WHO protocol as described before [13]. Prior to testing, each serum was treated with receptor-destroying enzyme (Cholera filtrate, Sigma, Germany) to inactivate non-specific inhibitors, achieving a final serum dilution of 1:10. The sera were then diluted serially twofold to detect titres up to  $\geq 1,280$ . All samples were tested twice to account for the variability of results in the HI assay.

### Statistical analysis

For statistical analyses, the following age groups were used: 18–29 (born 1979–92), 30–59 (born 1949–80) and  $\geq 60$  years (born before 1951) for adults and 0–9 (born 1994–2006), 10–13 (born 1990–96) and 14–17 years (born 1986–92) for children and adolescents. As end point we used the geometric mean of two antibody measurements as a single observation. We defined a titre  $\geq 40$  as seroprotective in line with other studies and with definitions used for evaluating influenza vaccines

**TABLE 1**

Independent variables investigated in the univariable logistic regression analysis among adults and children and adolescents, Germany, 2003–10

Variable name	Description	Population
Sex	Female, male	Adults, children and adolescents
Age group	18–29, 30–59, ≥60 years	Adults
	0–9, 10–13, 14–17 years	Children and adolescents
Sampling year	2008, 2009, 2010; 2008-09, 2010	Adults
	2003, 2004, 2005, 2006	Children and adolescents
Prior influenza vaccination	Seasonal or pandemic 2009 (based on vaccination card or self-reporting)	Adults
	Seasonal (based on vaccination card)	Children and adolescents
Number of influenza vaccinations	Based on vaccination card	Children and adolescents
Body weight	Body weight in kg	Adults, children and adolescents
Body mass index	Cut-off values: >25, >30, >33, >40, <18	Adults
Nationality	German vs non-German	Adults
Migrant	Migrant vs non-migrant	Children and adolescents
Geographical area	Former East-, West Germany (Berlin included in East Germany)	Children and adolescents
Smoking	Smoker; amount of smoking	Adults
	Participant is smoker; at least one parent is smoker; friends are smokers	Children and adolescents
Community size	Four categories for number of inhabitants; two categories for urban vs rural	Adults, children and adolescents
Social status	Three categories	Adults, children and adolescents
Education	Three categories	Adults
	Education of parents in three categories	Children and adolescents
Health status	Based on self-reporting	Adults, children and adolescents
Any chronic disease	Having any chronic disease	Adults, children and adolescents
Alcohol intake	Risk drinking; amount of alcohol	Adults
Number of persons living in household	Living alone vs 2, 3, 4, 5 or 6 persons	Children and adolescents
Sibling(s) in household	0 vs 1, 2, 3 or 4 siblings	Children and adolescents

(the 50% seroprotective threshold) [17]. Titres <10 were considered negative and were assigned a value of 5 for further calculations.

We calculated the proportion of persons with seroprotective titre and the geometric mean titre (GMT) with 95% confidence intervals (CI) in each age group for each influenza strain. For A/Wisconsin, we also calculated proportions with titres ≥10, ≥20, ≥80, ≥160 and ≥320. We examined the effect of different variables on the dichotomous variable 'titre ≥40', using logistic regression to obtain odds ratios (OR) with 95% CI for each influenza strain. As a first step, we conducted univariable regression analysis to identify potential factors associated with seropositivity. Independent variables with a p value <0.25 based on the Wald test were

considered for the multivariable analysis [18]. Manual stepwise forward selection based on p values from the Wald test was then used to obtain the final model. A p value < 0.05 was considered statistically significant. All variables in the final model were tested for possible two-way interactions. The variables investigated in the logistic regression analysis included demographic characteristics, history of influenza vaccination, year when blood sample was taken, region of residence in Germany, community size, factors related to health and social status and to living conditions (Table 1).

Statistical analyses for children and adolescents were conducted by using survey weights based on cross-classifications by age, sex, residence in Eastern or Western Germany and nationality (German vs non-German) to

**TABLE 2**

Haemagglutination inhibition antibody titres against influenza A(H3N2), by age group and virus strain, adults, Germany, 2008–2010 (n=600)

Age group (years)	n	Proportion with titre $\geq 40$ (95% CI)			GMT (95% CI)		
		A/Wisconsin	A/Perth	A/Niedersachsen	A/Wisconsin	A/Perth	A/Niedersachsen
18–29	200	59 (49–67)	24 (17–31)	23 (16–31)	33 (28–39)	12 (10–15)	15 (13–17)
30–59	200	30 (24–38)	16 (13–21)	49 (40–57)	17 (15–20)	9 (8–10)	27 (23–32)
$\geq 60$	200	42 (35–49)	23 (18–29)	26 (19–35)	23 (21–26)	14 (11–16)	18 (16–21)
<b>Overall</b>	<b>600</b>	<b>39 (34–44)</b>	<b>19 (17–22)</b>	<b>37 (31–43)</b>	<b>21 (19–23)</b>	<b>11 (10–12)</b>	<b>22 (19–24)</b>

CI: confidence interval; GMT: geometric mean titre.

Overall proportions and overall GMTs are weighted (see Methods).

account for differences between the design-weighted net sample and German population characteristics [15]. Original survey weights could not be used for the adult sample as the sera for our study were selected from a subgroup of the adult study population for which the survey weights were calculated. Based on German population characteristics of 2010 (data source: German Federal Statistical Office), we calculated weights based on sex and age group for adults, and these were used for the calculation of overall proportions and GMTs. In our study, 43 of the 180 study locations (sample points) of the original adult study were included. Two of 16 German states were not represented in our sample (the city states Bremen and Hamburg). As those city states comprise less than 5% of the total German population, we can assume that the adult sample was sufficient to represent German geographic characteristics [13]. The analyses accounted for the two-stage cluster design of the surveys.

All analyses were carried out with STATA software version 12.1 (StataCorp, College Station, TX, US). The study was approved by the Ethics Committee of Charité University Medicine, Berlin, Germany.

## Results

### Adults

The unweighted median age of the 600 participants included in the adult study was 47 years (range: 18–84) and 50% were female. Overall, 43% of participants (unweighted proportion) had been vaccinated at least once in their lifetime with an influenza vaccine (seasonal or pandemic 2009). Among the 600 adults, 44% had cross-reactive antibodies at titre  $\geq 40$  against A/Wisconsin. The overall prevalence of cross-reactive antibodies at titre  $\geq 40$ , weighted for sex and age group, was 39% (95% CI: 34–44) (Table 2).

For A/Wisconsin, the highest proportion of persons with titre  $\geq 40$  and the highest GMT were seen in the youngest age group (18–29 years). In the age group 30–59 years, the proportion was lower, whereas among persons aged 60 years and older, the proportion was

again higher, but did not reach the level of the youngest age group. For A/Perth, the pattern across the age groups was similar to A/Wisconsin with lower proportions in all age groups. For A/Niedersachsen, the proportions with seroprotective titre and the GMTs were highest in the age group 30–59 years.

The distribution of different cut-off values for antibody titres against A/Wisconsin in the three age groups was explored in reverse cumulative distribution curves (Figure 1). The pattern of difference between age groups was observed at all cut-off values. Titres declined rapidly after titre 40, and no titres  $> 320$  were measured.

The final multivariable logistic regression model for all three strains contained age group, sex and prior influenza vaccination as independent variables (Table 3). Sex was included in the final model to show that it was not associated with seroprotective titres; inclusion did not relevantly change ORs for other variables. Those 60 years and older had higher odds of having a seroprotective titre compared with the 30–59 year-olds in the univariable analysis for A/Wisconsin (OR: 1.6; 95% CI: 1.1–2.4;  $p=0.014$ ). This difference was not significant after adjustment for prior influenza vaccination. Prior influenza vaccination was shown to be a factor associated with seroprotective titre for A/Wisconsin and A/Perth with the highest OR for A/Perth and lower for A/Wisconsin; the association for A/Niedersachsen was not significant. There were no significant interactions between the variables of the final models.

### Children and adolescents

The unweighted median age of persons in this study was 13 years (range: 2–17) and 46% were female. Overall, 9.6% of the study participants (unweighted proportion) had been vaccinated at least once in their lifetime with a seasonal influenza vaccine. Among the 815 children and adolescents, 42% had cross-reactive antibodies at titre  $\geq 40$  against A/Wisconsin. The weighted overall prevalence of cross-reactive antibodies at titre  $\geq 40$  was 41% (95% CI: 37–45) (Table 4). For the three influenza strains investigated, the lowest proportion with seroprotective titre and the lowest

**TABLE 3**

Adjusted effects of associated factors on the odds of haemagglutination inhibition antibody titre  $\geq 40$  against influenza A(H3N2), by virus strain, adults, Germany, 2008–2010 (n = 582)

Variable	A/Wisconsin			A/Perth			A/Niedersachsen		
	OR	95% CI	p value	OR	95% CI	p value	OR	95% CI	p value
Age group (years)									
18–29	REF	NA	NA	REF	NA	NA	REF	NA	NA
30–59	0.28	0.16–0.50	<0.001	0.53	0.32–0.90	0.019	3.2	1.9–5.4	<0.001
$\geq 60$	0.36	0.20–0.64	0.001	0.53	0.31–0.90	0.019	1.0	0.61–1.70	0.954
Sex									
Male	REF	NA	NA	REF	NA	NA	REF	NA	NA
Female	1.2	0.85–1.80	0.320	0.97	0.63–1.50	0.903	1.2	0.78–1.80	0.434
Prior influenza vaccination	2.4	1.7–3.4	<0.001	4.3	2.6–7.2	<0.001	1.5	0.99–2.20	0.055

CI: confidence interval; NA: not applicable; OR: odds ratio; REF: reference group. Participants with missing data (n = 18) were excluded.

**TABLE 4**

Haemagglutination inhibition antibody titres against influenza A(H3N2), by age group and virus strain, children and adolescents, Germany, 2003–2006 (n = 815)

Age group (years)	n	Proportion with titre $\geq 40$ (95% CI)			GMT (95% CI)		
		A/Wisconsin	A/Perth	A/Niedersachsen	A/Wisconsin	A/Perth	A/Niedersachsen
0–9	62	15 (7–30)	2.2 (0.5–9.4)	0.6 (0.0–4.2)	13 (10–17)	6.1 (5.5–6.8)	5.7 (5.1–6.3)
10–13	353	37 (31–44)	3.0 (1.6–5.5)	3.6 (2.0–6.5)	21 (19–24)	6.3 (6.0–6.7)	7.9 (7.4–8.5)
14–17	400	47 (42–52)	3.9 (2.3–6.5)	12.0 (9.0–16.0)	27 (24–29)	6.5 (6.1–6.9)	11 (10–12)
<b>Overall</b>	<b>815</b>	<b>41 (37–45)</b>	<b>3.4 (2.3–5.0)</b>	<b>8.1 (6.3–10.0)</b>	<b>23 (22–25)</b>	<b>6.4 (6.1–6.7)</b>	<b>9.3 (8.8–9.8)</b>

CI: confidence interval; GMT: geometric mean titre. Proportions and GMTs are weighted (see Methods).

GMT were seen among those younger than 10 years, with proportions and GMTs increasing with increasing age. The overall proportion with seroprotective titre for A/Perth and A/Niedersachsen was less than 10%.

The distribution of different cut-off values for antibody titres against A/Wisconsin in the three age groups was explored using reverse cumulative distribution curves (Figure 2). The pattern of difference between age groups was observed at all cut-off values, with rapidly declining titres after titre 40 and no measurable titres  $> 320$ .

The final multivariable logistic regression model included age group, sex and prior influenza vaccination as independent variables for all three strains (Table 5). For A/Perth, there was no significant association with age. Significant associations between prior vaccination and seroprotective titres were shown for each tested

strain, with the highest OR for A/Perth, followed by A/Wisconsin and A/Niedersachsen. Female sex was associated with higher odds of having a seroprotective titre for A/Wisconsin and A/Niedersachsen. There were no significant interactions between the variables of the final models.

## Discussion

Using sera from two representative health examination surveys in the German population including adults, children and adolescents, we have shown that children younger than 10 years (in 2003–06, i.e. those born 1994–2006) had the lowest prevalence of cross-reactive antibodies against the A(H3N2)v virus representative A/Wisconsin. Among children aged 10 years and older, adolescents and adults, the prevalence was markedly higher. The pattern of differences between age groups could be observed at many titre cut-off values, not just at the level defined as seroprotective. The observed



**TABLE 5**

Adjusted effects of associated factors on the odds of haemagglutination inhibition antibody titre  $\geq 40$  against influenza A(H3N2), by virus strain, children and adolescents, Germany, 2003–2006 (n = 815)

Variable	A/Wisconsin			A/Perth			A/Niedersachsen		
	OR	95% CI	p value	OR	95% CI	p value	OR	95% CI	p value
Age group (years)									
0–9	REF	NA	NA	REF	NA	NA	REF	NA	NA
10–13	3.2	1.3–8.0	0.013	1.3	0.27–6.60	0.713	5.7	0.69–47.0	0.105
14–17	4.6	1.8–12.0	0.001	1.8	0.40–7.90	0.442	20.0	2.6–151	0.004
Sex									
Male	REF	NA	NA	REF	NA	NA	REF	NA	NA
Female	1.5	1.0–2.0	0.025	0.78	0.33–1.80	0.549	2.7	1.5–4.9	0.001
Prior influenza vaccination	3.4	1.8–6.5	<0.001	5.4	2.2–14.0	<0.001	2.2	1.1–4.6	0.028

CI: confidence interval; NA: not applicable; OR: odds ratio; REF: reference group.  
The regression models are weighted (see Methods).

pattern of age-specific seroprevalence of cross-reactive antibodies against the A(H3N2)v virus is consistent with findings from countries in Asia (Japan), Europe (Norway, UK) and North America (Canada, US) [7–11]. The levels of cross-reactive antibody titres against the influenza A(H3N2)v virus in the different age groups were consistent with the likelihood of exposure to seasonal A/Wuhan/359/95-like influenza strains. Likewise, cross-reactive antibody titre levels against the contemporary human influenza A(H3N2) virus (A/Perth) and the swine influenza A(H3N2) virus (A/Niedersachsen) were in line with the likelihood of exposure to related circulating seasonal influenza strains and confirm the specificity of our results regarding the A(H3N2)v virus.

Several mechanisms may explain the observed prevalences of cross-reactive antibodies against the A(H3N2)v virus and the highest proportions with seroprotective titre among those aged 14–17 and 18–29 years. Young adults are at high risk for infection as their contact patterns result in frequent exposure to circulating seasonal influenza viruses. The 14–29 year-olds in our study are likely to have been infected at a young age with the A/Wuhan/359/95-like human A(H3N2) virus that is very similar to A(H3N2)v. This may have represented the initial exposure to influenza viruses, resulting in long-lasting immunity. The first infection with an influenza virus is believed to influence and dominate immune responses to later infections (original antigenic sin theory) [19,20]. Although they had the opportunity of exposure to A/Wuhan/359/95-like human A(H3N2) viruses, older adults had lower antibody levels against the A(H3N2)v virus. Lower frequency of influenza infections among older adults may explain this observation. In addition, the initial childhood exposure of these adults to influenza was to viruses different from the A(H3N2)v virus. Children younger than 10 years were

least likely to have been exposed to influenza viruses similar to the A(H3N2)v virus, which explains why the antibody levels in this group were the lowest.

Any influenza vaccination in the lifetime of our study participants (after adjustment for age and sex) was positively associated with having a seroprotective titre of cross-reactive antibodies against all three investigated strains. This effect was strongest for the human influenza A(H3N2) virus A/Perth and weaker for the swine-related influenza A(H3N2) viruses A/Wisconsin and A/Niedersachsen, which is plausible considering that A/Wisconsin and A/Niedersachsen are less related to human vaccine strains than A/Perth.

In the final model, female children and adolescents had slightly higher odds of having a seroprotective titre against the A(H3N2)v and swine A(H3N2) virus. This may be explained by a generally stronger immune response to infections and vaccination in women, and sex-related differences in HI titres after seasonal influenza vaccination have been described before [21]. Such an effect was not observed among adults, nor with the human A(H3N2) virus strain among children and adolescents.

The proportions of adults with seroprotective titre against the A(H3N2)v virus differed significantly between the age groups 30–59 and  $\geq 60$  years. Similar observations have been made by others for the A(H3N2)v virus [10,11] and also for seasonal H3N2 and H1N1 subtypes where, among others possible causes, the priming infection of a person and prior influenza vaccination were suggested to be strong determinants of such a pattern [20,22–24]. Using information about prior influenza vaccination in a multivariable logistic regression model, we have shown that, in our

population, this difference was most probably a result of the higher proportion of elderly people with prior influenza vaccination (one of the risk groups for whom seasonal influenza vaccination is recommended in Germany).

Our study had several limitations. Serum samples originated from two different population surveys carried out in different time periods over several years (2003–06 and 2008–10) and therefore under the influence of different circulating human influenza viruses. Considering this and the exposure history of the population after the surveys were carried out, caution should be applied when using our results to predict age-related susceptibility in the population today. Some of the age groups of KiGGS and DEGS participants overlap when they are considered as birth cohorts. This overlap may explain the similar prevalence of seroprotective titres among those aged 14–17 and 18–29 years.

HI is used as a correlate for immunity against infection with influenza virus, but beside cross-reactive antibodies, many other biological mechanisms influence the actual immune response of a person (e.g. T-cell mediated immunity) [25,26] and thus susceptibility to influenza viruses. Increased HI titres may not necessarily prevent higher infection rates. We demonstrated this in an earlier study conducted in Germany, where the age group with the highest pre-pandemic titres against the A(H1N1)pdm09 virus had the highest infection rates with this virus [13]. It cannot be assumed that prior influenza vaccination necessarily provides true protection against infection with the A (H3N2)v virus as studies have shown that prior vaccination against seasonal influenza may be associated with a higher risk of becoming infected with a pandemic influenza strain (A(H1N1)pdm09) [27–30]. A recent study did not show the 2011/12 seasonal trivalent inactivated influenza vaccine to have a cross-protective effect against the A(H3N2)v virus in seronegative ferrets [31]. This may have implications for young children not yet infected by influenza virus because a seasonal influenza vaccine without an A(H3N2)v virus component would most probably not confer any immunity against the A(H3N2)v virus among them. In addition, the assumption that an HI titre  $\geq 40$  indicates 50% seroprotection is based on studies in adults. Recent studies suggest that a titre  $\geq 40$  or higher may be used as a correlate of 50% seroprotection for children aged 6–17 years [32], but higher titres may be needed to predict at least 50% protection in younger age groups [33]. Furthermore, compared to younger adults, HI titres seem to be less reliable in predicting protection in the elderly and in certain risk groups [34].

Our results indicate that young children have the highest susceptibility to infection with the A(H3N2)v virus. In case of increased sustained human-to-human transmission of the virus, these children may contribute to the rapid spread of the A(H3N2)v virus as transmission among children plays a major role in the propagation of

the spread of influenza [35]. They are also at high risk for severe disease from influenza infections. A large proportion of the population 10 years and older has cross-reactive antibodies at potentially seroprotective level and thus, based on this correlate for immunity, may be at low risk for A(H3N2)v infection. Our results suggest that the first exposure to circulating seasonal influenza viruses in a person's lifetime may predict their long-term cross-reactive antibody response. These cross-reactive antibodies can be boosted by vaccination and possibly by exposure to seasonally circulating influenza viruses. Further studies may further our understanding of the effect of seasonal influenza viruses on the serological immune response.

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### Conflict of interest

None declared.

### Authors' contributions

B. Blümel contributed to the overall design of the study, analysed the data, and drafted the manuscript. B. Schweiger contributed to the study design, supervised the laboratory analysis, and reviewed the manuscript. M. Dehnert and I. Czogiel contributed to the design of the analysis, supervised data analysis, and reviewed the manuscript. S. Buda, A. Reuss contributed to the study design and reviewed the manuscript. P. Kamtsiuris, M. Schlaud, C. Poethko-Müller, and M. Thamm provided the data and sera for the study and reviewed the manuscript. W. Haas contributed to the overall design of the study and the design of the analysis, and reviewed the manuscript.

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