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Influenza B virus infections in Western Saxony, Germany in three consecutive seasons between 2015 and 2018: Analysis of molecular and clinical features

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ABSTRACT

Background: The impact of annual influenza epidemics and prevailing strains varies worldwide and regional. The majority of vaccines used contained two influenza A strains and only one influenza B strain (trivalent vaccine).
Aim: The aim of the study was to compare laboratory confirmed influenza B cases during three consecutive years with respect to vaccination history, clinical symptoms and molecular virology.
Methods: Partial HA gene sequences were analyzed for lineage determination and complete HA sequence in cases with reported vaccination and in fatal cases. Clinical data were retrieved from patient charts.
Findings: During the 2015/16 season, 75 influenza B cases were retrieved; 11 in 2016/17, and 274 in 2017/18. The frequency of Yamagata-lineage strains increased from 7.6% to 100%. No difference was detected in the relative frequency of co-morbidities in season 2017/18. 37.7% of the adult patients and 4.5% of pediatric patients were vaccinated against influenza.
Interpretation: Phylogenetically, Yamagata strains clustered similarly in 2017/2018 when compared to the previous two influenza seasons. While the relative frequency of influenza B cases differed, the clinical symptoms remained similar.
Conclusion: World Health Organization recommendations for the use of tetravalent vaccines that contain two influenza B strains (Yamagata and Victoria) in addition to the two influenza A strains (H1N1 and H3N2) should be implemented in national vaccination guidelines.
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1. Introduction

Influenza B virus of the *Orthomyxoviridae* family [1] has a segmented single stranded, negative sense RNA genome. First isolated in 1940, influenza B virus diverged into two lineages, Victoria and Yamagata, in the late 1970s with similar clinical [2] but different phylodynamic properties [3]. Although infections in pigs and seals were observed, there is no known animal reservoir for influenza B [4]. Thus, while influenza A infections can be zoonotic, influenza B virus only circulates in the human population. The narrow host range and slower evolution [5] are thought to be the main contributing factors to the less frequent occurrence of influenza B epidemics [6].

In 2017/18 influenza B predominance was reported in countries throughout the northern hemisphere. It was the leading influenza type in Europe and Canada [7–9] and the second most common type after influenza A/H3N2 in the USA [10]. In Germany, after the seasons of 2001/2, 2005/6 and 2015/16, it was only the fourth season of the 21st century with a dominance of influenza B [11]. As the prevailing lineage of influenza B changes frequently, the World Health Organization (WHO) recommended to include both lineages in a tetravalent vaccine in 2012 [12].

The key component of the vaccine is the viral hemagglutinin (HA) that covers the viral surface in a trimeric form. The major antigenic regions are the 120-loop, 150-loop, 160-loop, and 190-helix [13] and are located on the HA1 subunit which forms the globular head domain. It is responsible for recognition and binding of sialic acids on the surface of the target cells. The HA2 subunit is the main component of the HA stalk. Although some antibody

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cross reactivity is observed [14,15], vaccine efficiency between the two lineages might be reduced in seasons in which the formulation of the trivalent influenza vaccine does not match the circulating strain due to antigenic differences [16,17]. Additionally, there is growing evidence that vaccine effectiveness is already waning within a season thus reducing immunological protection even if the vaccine contained the matching strain in the previous season [18].

The aim of this study was to analyze clinical and molecular features of laboratory-confirmed influenza B cases during three consecutive seasons between 2015 and 2018. A comparison between patient characteristics was done in relation to the combined seasons of 2015/16 and 2016/17 when appropriate. Additionally, molecular epidemiology of the viral hemagglutinin (HA) gene was performed on selected isolates and amino acid changes were mapped to the major antigenic domains of the HA protein.

2. Methods

Specimens and clinical data. 11,956 respiratory samples, including nasal aspirates, supernatants from nasal and pharyngeal swabs, throat rinsing fluid, tracheal secretions, and bronchoalveolar lavage fluids, of 7681 patients were tested for respiratory virus infections. Testing was initiated at the discretion of the

Table 1
Total and relative numbers of influenza types per season.

Season	*Influenza B* [n (%)]		*A/H3N2* [n (%)]		*A/H1N1* [n (%)]		*Total* [n]
2015/2016	75	(39.9)	13	(6.9)	**100**	**(53.2)**	188
2016/2017	11	(4.9)	**211**	**(93.4)**	4	(1.8)	226
2017/2018	**274**	**(76.1)**	20	(5.6)	66	(18.3)	360

Bold numbers represent the dominant influenza type of the respective season.

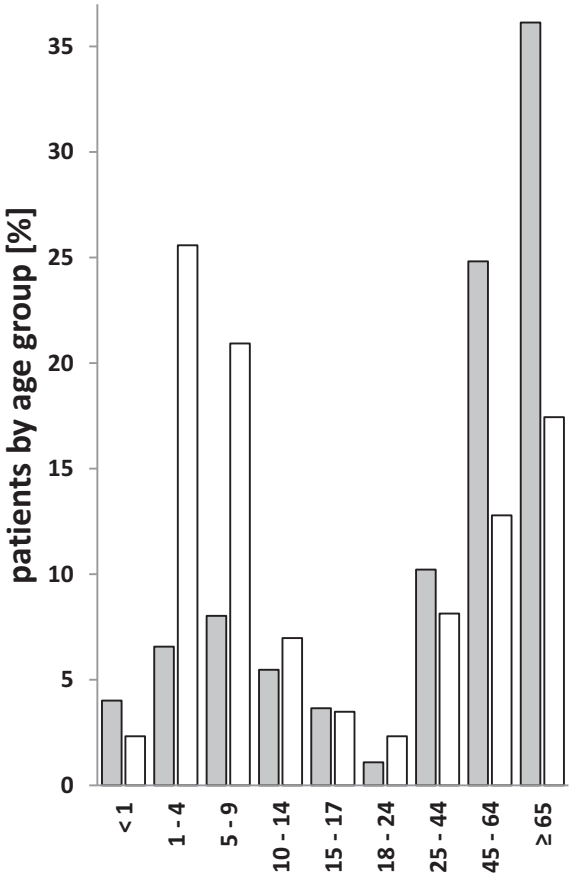

Fig. 2. Relative distribution of influenza B cases by age. Season 2017/18 is represented by grey bars and the combined seasons of 2015/16 and 2016/17 are shown in white bars.

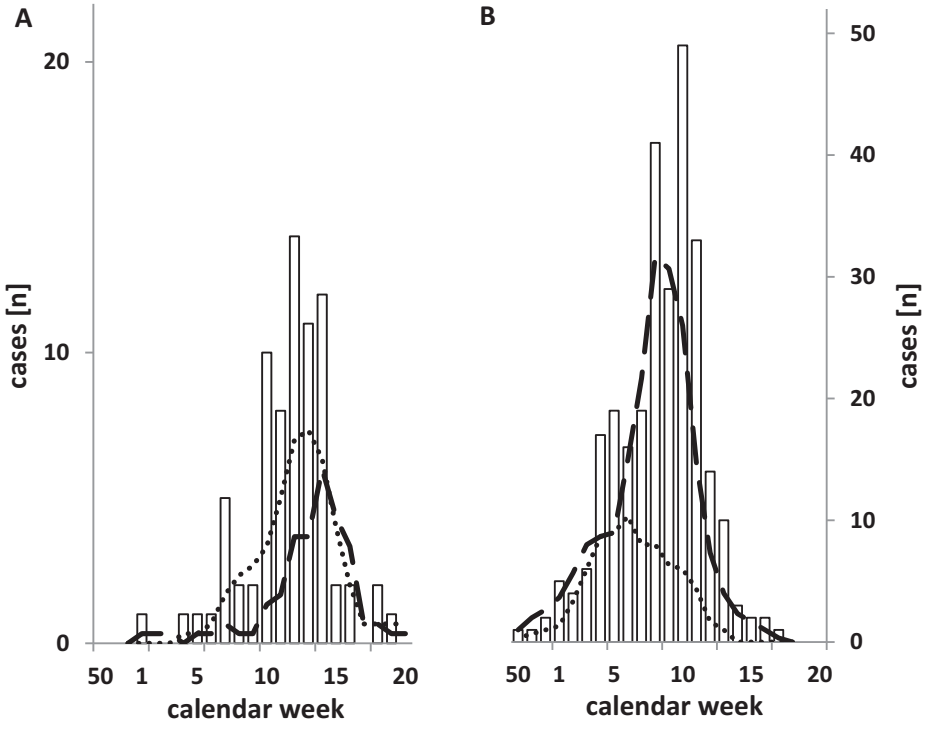

Fig. 1. Number of patients with laboratory-confirmed diagnosis of influenza B per week (columns) for season 2015/16 (A) and 2017/18 (B). The dotted lines represent the case numbers for patients under 18 years of age and the dashed lines patients that were 18 years of age and older.

treating physician. Follow-up samples that were tested positive within six weeks of the initial detection were treated as a single case. Clinical data were retrieved retrospectively from patient charts. A season was defined as starting on the 1st of October of one year and ending on the 30th of September of the following year.

Nucleic acid (NA) extraction. Total NA was extracted from 200 μl of respiratory samples using the DNA and Viral NA Small Volume Kit and the MagNA Pure 96 instrument (Roche, Mannheim, Germany) according to the manufacturer's instructions (output volume 100 μl). The NA samples were used immediately for respiratory virus detection and stored in aliquots at −80 °C until further use.

Influenza B virus detection. Clinical samples were tested with a commercially available test for respiratory viruses (NxTAG RPP, Luminex corporation, Austin, Texas, USA) according to the manufacturer's instructions. The panel included influenza viruses A and B, respiratory syncytial virus A and B, parainfluenza viruses 1–4, human coronavirus (including 229E, NL63, OC43 and HKU1), human metapneumovirus, adenovirus, human bocavirus, rhinovirus, and enterovirus.

Lineage determination. NA sequences of a 123 bp-long amplicon of the HA gene [19] were assessed (BigDye Terminator Sequencing Kit v1.1 and ABI 3500 Genetic Analyzer, Applied Biosystems, Foster City, USA). The sequenced region corresponds to nucleotide positions 966–1088 and 963–1085 of the complete HA gene of the Victoria lineage (B/Brisbane/60/2008) and the Yamagata lineage (B/Phuket/3073/2013), respectively. Sequences with nucleotides G16, G17, G28, G55, C64, G73, and A112 belonged to the Victoria-lineage. Viruses with nucleotides A16, A17, A28, A55, T64, T73, and G112 belonged to the Yamagata-lineage.

Phylogenetic analysis. NA sequencing of the complete HA was performed according to WHO guidance [20]. In cases of low influenza B concentration an alternative protocol generating three instead of two overlapping fragments was used [21]. Phylogenetic trees of the 1710 nucleotides large HA coding region were constructed at nucleotide level with the MEGA software version 6 using the Maximum-Likelihood method. Bootstrap analysis was performed with 1000 replicates [22]. Complete HA sequences were submitted to Genbank (accession numbers MK459565 - MK459656).

Statistical analysis. Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 24.0 (Armonk, NY: IBM Corp.). Continuous values were expressed as mean or median (range) and categorical data as frequencies (percentages). Student's *t*-test or Mann-Whitney *U* test was performed to compare means. Chi-square or Fisher's exact test were performed for categorical variables. All tests were two-tailed. A p-level of < 0.05 was considered significant.

3. Results

3.1. Weekly detection of influenza B cases

Relative and absolute numbers of influenza types detected between 2015 and 2018 are shown in Table 1. For season 2015/16 the maximum number of cases occurred between weeks 10 and 14 of 2016. The peak occurrence in patients below 18 years was in week 12 while it was week 14 for the adult patients (Fig. 1A). The eleven cases of the season of 2016/17 occurred

Table 2
Study population and clinical features of influenza B infected cases.

		(A) paediatric patients			(B) adult patients			(C) all seasons		
		2015 - 2017	2017/2018	*p* -value	2015 - 2017	2017/2018	*p* -value	<18	≥18	*p* -value
study population										
age [years]	*[median (range)]*	5 (0 - 17)	7 (0-17)	0.29	61 (19-86)	64.5 (18-98)	0.1	6 (0-17)	64 (18-98)	**<0.001**
female gender	*[% (n/total)]*	51 (26/51)	60.5 (46/76)	0.36	22.9 (8/35)	55.6 (110/198)	**<0.001**	56.7 (72/127)	50.6 (118/233)	0.32
inpatients	*[% (n/total)]*	92.2 (47/51)	85.5 (65/76)	0.28	80 (28/35)	73.2 (145/198)	0.42	88.2 (112/127)	74.2 (173/233)	**0.003**
length of stay [days]	*[median (range)]*	4 (0-33)	3.5 (0-143)	0.72	5 (0-37)	7 (0-76)	0.45	4 (0-143)	7 (0-76)	**0.02**
vaccination										
no	*[% (n/total)]*	92.5 (37/40)	97.2 (70/72)		82.6 (19/23)	65.2 (88/135)		95.5 (107/112)	67.7 (107/158)	
trivalent	*[% (n/total)]*	5 (2/40)	2.8 (2/72)	0.41	17.4 (4/23)	25.9 (35/135)	0.2	3.6 (4/112)	24.7 (39/158)	**<0.001**
tetravalent	*[% (n/total)]*	2.5 (1/40)	0 (0/72)		0 (0/23)	8.9 (12/135)		0.9 (1/112)	7.6 (12/158)	
comorbiditis/risk factors										
asthma	*[% (n/total)]*	2 (1/51)	2.7 (2/75)	1	2.9 (1/34)	3.9 (7/179)	1	2.4 (3/126)	3.8 (8/213)	0.55
COPD	*[% (n/total)]*	0 (0/51)	0 (0/75)	-	23.5 (8/34)	19.6 (35/179)	0.64	0 (0/126)	20.2 (43/213)	**<0.001**
chronic kidney failure	*[% (n/total)]*	0 (0/51)	1.3 (1/75)	1	41.2 (14/34)	38.2 (68/178)	0.85	0.8 (1/126)	38.7 (82/212)	**<0.001**
cardiac insufficiency	*[% (n/total)]*	0 (0/51)	1.3 (1/75)	1	20.6 (7/34)	14.6 (26/178)	0.44	0.8 (1/126)	15.6 (33/212)	**<0.001**
diabetes	*[% (n/total)]*	0 (0/51)	4 (3/75)	0.27	23.5 (8/34)	29.8 (54/181)	0.54	2.4 (3/126)	28.8 (62/215)	**<0.001**
structural lung disease	*[% (n/total)]*	2 (1/51)	8 (6/75)	0.24	8.8 (3/34)	12.3 (22/179)	0.77	5.6 (7/126)	11.7 (25/213)	0.08
malignancy	*[% (n/total)]*	0 (0/51)	4 (3/75)	0.21	50 (17/34)	33 (59/179)	0.08	2.4 (3/126)	35.7 (76/213)	**<0.001**
immunosuppression	*[% (n/total)]*	0 (0/51)	2.7 (2/75)	0.51	35.3 (12/34)	30.4 (55/181)	0.69	1.6(2/126)	31.2 (67/215)	**<0.001**
at least one of the above	*[% (n/total)]*	3.9 (2/51)	18.4 (14/76)	**0.026**	80 (28/35)	75.3 (149/198)	0.67	12.6 (16/127)	76 (177/233)	**<0.001**
clinical presentation and features										
fever										
yes	*[% (n/total)]*	70.6 (36/51)	86.4 (57/66)		31 (9/29)	50.4 (62/123)		79.5 (93/117)	46.7 (71/152)	
subfebrile	*[% (n/total)]*	9.8 (5/51)	1.5 (1/66)	0.55	20.7 (6/29)	6.5 (8/123)	0.3	5.1 (6/117)	9.2 (14/152)	**<0.001**
no	*[% (n/total)]*	19.6 (10/51)	12.1 (8/66)		48.3 (14/29)	43.1 (53/123)		15.4 (18/117)	44.1 (67/152)	
leukocyte count [exp 9/l]	*[median (IQR)]*	6.9 (4.9-10.2)	8.1 (4.5-10.5)	0.86	7.5 (4.7-9.3)	8.9 (4.1-10.4)	0.32	7.7 (4.7-10.5)	6.8 (4.2-9.7)	0.33
cough	*[% (n/total)]*	70.6 (36/51)	57.6 (38/66)	0.18	65.5 (19/29)	68.9 (91/132)	0.83	63.2 (74/117)	68.3 (110/161)	0.44
new dyspnoea	*[% (n/total)]*	19.6 (10/51)	18.2 (12/66)	1	25.8 (8/31)	39.6 (59/149)	0.16	18.8 (22/117)	37.2 (67/180)	**0.001**
ARDS	*[% (n/total)]*	0 (0/51)	0 (0/75)	-	5.9 (2/34)	4.9 (9/185)	0.68	0 (0/126)	5 (11/219)	**0.009**
ICU stay	*[% (n/total)]*	9.8 (5/51)	10.5 (8/76)	1	20 (7/35)	25.8 (51/198)	0.53	10.2 (13/127)	24.9 (58/233)	**0.001**
invasive ventilation	*[% (n/total)]*	0 (0/51)	2.6 (2/76)	0.51	17.1 (6/35)	12.6 (25/198)	0.43	1.6 (2/127)	21.4 (31/233)	**<0.001**
fatal outcome	*[% (n/total)]*	0 (0/51)	1.3 (1/76)	1	11.4 (4/35)	11.1 (22/198)	1	0.8 (1/127)	11.2 (26/233)	**<0.001**
use of neuraminidase inhibitors	*[% (n/total)]*	0 (0/51)	1.3 (1/75)	1	11.4 (4/35)	10.6 (19/180)	0.77	0.8 (1/126)	10.7 (23/215)	**<0.001**
nosocomial infection	*[% (n/total)]*	0 (0/51)	5.3 (4/76)	0.15	17.1 (6/35)	17.9 (35/196)	1	3.1 (4/127)	17.7 (41/231)	**<0.001**
viral coinfection	*[% (n/total)]*	15.7 (8/51)	14.5 (11/76)	1	17.1 (6/35)	4.5 (9/198)	**0.014**	15 (19/127)	6.4 (15/233)	**0.009**

Analyzed categories are displayed on the column to the left and either given as frequencies [%], median and range [median (range)] or median and interquartile range [median(IQR)]. (n/total) indicates the respective cases of the total amount of available data. A comparison was done for the pediatric (A) and adult (B) population between the combined seasons of 2015/16 and 2016/17 (2015–2017) and the season of 2017/18. In (C), a comparison was done for the whole study period between adult and pediatric cases.

between week 48 of 2016 and week 21 of 2017. No case maximum was observed, however, there were no cases between weeks 50 of 2016 and six of 2017 (data not shown). For season 2017/18 the maximum number of cases occurred between weeks 8 and 11 of 2018. The peak occurrence in patients under 18 was in week 8 while it was week 10 for the adult patients (Fig. 1B). Positive results for all tested respiratory viruses are shown in supplementary Fig. S1.

3.2. Study population and clinical features

A total of 360 laboratory-confirmed influenza B cases were included in the study and were derived from 75 patients in 2015/16, eleven patients in 2016/17, and 274 patients in 2017/18. Cases of seasons 2015/16 and 2016/17 were combined (i.e. 2015–2017) and compared to 2017/18. The two groups differed in the age distribution ($p < 0.001$; Fig. 2). Patients of the season 2017/18 were older than patients of 2015–2017. The median age was 56 and 10 years, respectively. However, when considering the pediatric (patients 17 and younger) and adult (patients 18 and older) populations separately, median age and age range did not differ between groups 2015–2017 and 2017/18. Therefore, a further analysis was done to compare the clinical features of the influenza B infections of the pediatric and adult patients with regard to the seasons and between the two age groups (Table 2). For the pediatric patients there were no significant differences between season 2017/18 and 2015–2017 except for the combined rate of comorbidities and risk factors for a severe course of the influenza. However, this could not be attributed to a single analyzed factor. When looking at the adult population, there were no significant differences between season 2017/18 and the previous seasons except for a higher proportion of female patients and a lower proportion of viral coinfections in 2017/18. Most differences were noted between the age groups. 32.3% of the adult patients were vaccinated, mostly with the trivalent vaccine, whereas 95.5% of the pediatric patients were unvaccinated. The rate of comorbidities was significantly higher in the adult population. Furthermore, the clinical course of the disease was more severe in the adult population as higher rates of ICU admissions, invasive ventilation, and fatal outcomes were observed.

3.3. Phylogenetic analysis

Lineage Determination. Sufficient material for the phylogenetic lineage determination of the detected influenza B viruses was available in 337 (93.6%) of the cases, including 71 of season 2015/16, 10 of season 2016/17, and 256 of season 2017/18. Influenza B strains of the Victoria-lineage predominated in season 2015/16 (93%, n = 66/71), were rare in season 2016/17 (10%, n = 1/10), and were not detected in season 2017/18. Influenza B strains of the Yamagata-lineage were rare in season 2015/2016 (7%, n = 5/71), but prevailed in 2016/17 (90%, n = 9/10) and 2017/18 (100%).

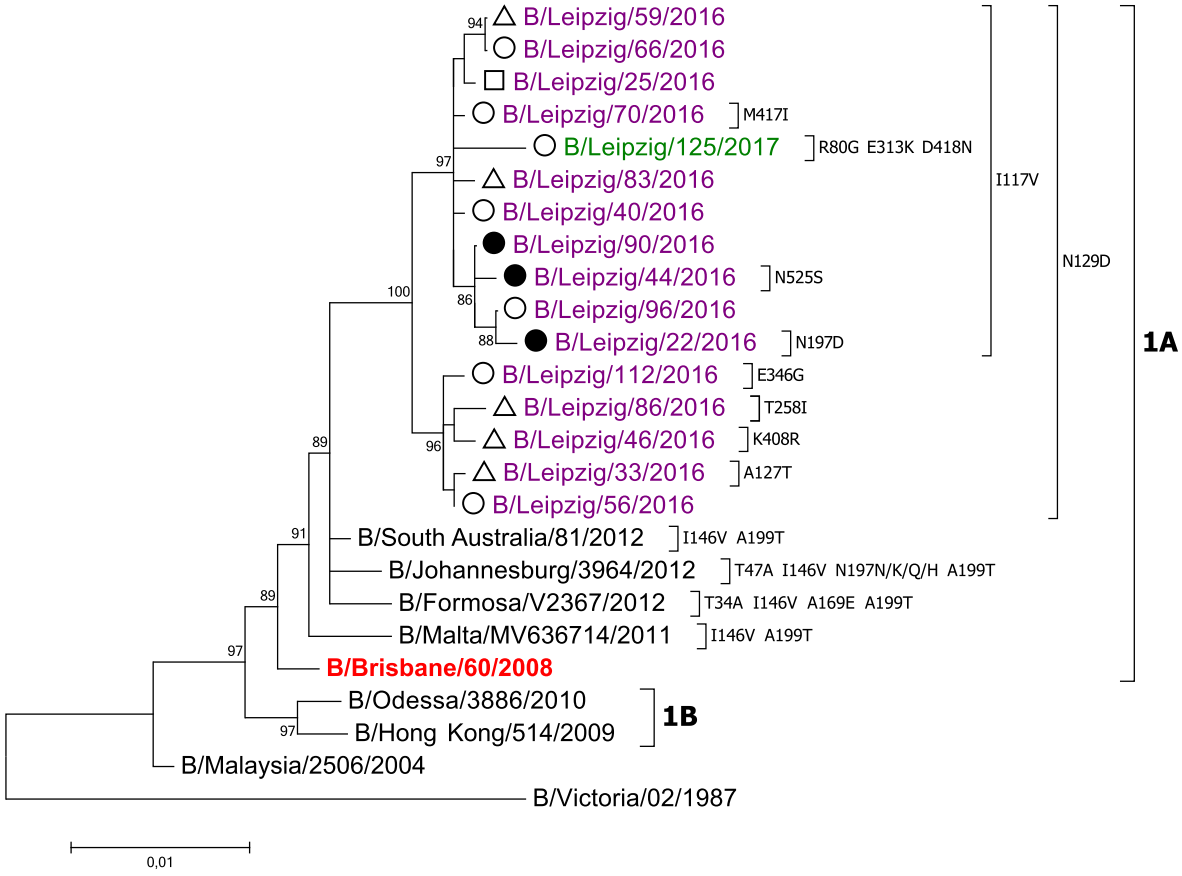

Fig. 3. Phylogenetic trees of the hemagglutinin (HA) nucleotide sequences of (A) Victoria- and (B) Yamagata-like influenza B viruses. Trees were constructed based upon nucleic acid alignments of the complete coding sequences (1710 nucleotides) of HA gene (1755 nucleotides) by the Maximum-Likelihood method. Numbers at each node represent bootstrap values obtained with 1000 replicates (values > 75 are shown). Reference strains are given at the bottom of the trees (black). Isolates from this study are labeled with the following symbols: vaccinated with trivalent vaccine (triangle), vaccinated with tetravalent vaccine (square), randomly selected case without vaccination history (circle), fatal cases (black filled symbols). Strains of season 2015/16 are shown in purple, strains of season 2016/17 are shown in green and strains of season 2017/2018 are shown in blue. Amino acid changes with regard to the vaccine strain (red) are given in brackets. The clades of the detected lineages are shown on the right (numbers). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

HA phylogeny. Sequence analysis of the whole hemagglutinin gene was performed for 16 Victoria and 76 Yamagata strains and consistently confirmed the results of the initial lineage determination. Included were all strains of patients with a known trivalent (n = 33) or tetravalent (n = 7) vaccination, randomly selected patients without known vaccination (n = 51), and of all patients that had a fatal outcome within 30 days of the influenza B detection (n = 27). Except for one strain of 2017, all Victoria strains originated from the 2015/2016 season and belonged to the 1A clade. All of the strains were phylogenetically closely related and did not cluster based on vaccination history or fatal outcome (Fig. 3A). All analyzed Yamagata strains belonged to clade 3. All of the strains were phylogenetically closely related, but strain phylogeny indicates a discrete drift from season 2015/16 to season 2017/18. Again, no clustering with regard to vaccination history or fatal outcome was observed (Fig. 3B).

Amino acid changes. The detected amino acid changes (AA-changes) within the HA protein are depicted in Table 3 (HA0 numbering). In comparison to the vaccine strain B/Brisbane/60/2008, that is used as a reference, all Victoria strains showed a distinct amino acid at position 129 (N129D). Furthermore, eleven strains showed the I117V substitution including the single strain of season 2016/17. Both AA-changes are located within the 120-loop region. Further AA-changes were only found in single strains (A127T, N197D, T258I, E346G, K408R, M417I, and N525S, respectively). The strain of season 2016/17 showed three AA-changes (R80G, E313K, and D418N). In comparison to the vaccine strain B/Phuket/3073/2013, all Yamagata strains showed a distinct amino acid at positions 251 (M251V) and 172 (L172Q). Following AA-changes were found in up to five of the strains: H14Y, V15M, V16I, P31Q, T35A, T37T/K, A42S, R69M, M71T, V73M, D126N, I150V, Y165H, T181A, E182K, N217D, D229G, D232N, K253S, R278Q, I438M, A505V, and D529N, respectively.

4. Discussion

The influenza season of 2017/18 was dominated by the Yamagata-lineage of influenza B in Europe. In Germany, it was the largest season in terms of case numbers since the establishment of the nationwide surveillance system in 2001 [11]. Large scale epidemics or pandemics are usually caused by influenza A. This is attributed mostly to the broader host range and faster rate of evolution of influenza A [6]. Influenza B infections usually occur with a temporal delay towards the end of an influenza A-dominated season [23]. The exceptional impact of influenza B infections in 2017/18 with regard to public health was therefore surprising. However, seasons with a high proportion of influenza B infections occur infrequently. Specifically, in Germany, the mild seasons of 2001/2 and 2005/6 and the severe season of 2015/16 have been dominated by influenza B with a share of up to 78% of all influenza cases. The high severity of the season 2017/18 with an overall estimate of 9 million excess consultations in Germany [11] may be attributed to several factors.

According to the ECDC, the influenza season of 2017/18 started earlier than most of previous ones [9]. While this is true for the epidemic phase of the two influenza B heavy seasons in this study, the time frame for isolated cases was comparable. In the season of 2015/16 the epidemic phase started in week 5 of 2016 and lasted until week 15. In 2017/18 the epidemic threshold was crossed in week 52 of 2017 until week 14 of 2018 [11].

Another factor contributing to the severity of the season might be the patient population itself. Both the attack rate and the relative illness ratio of influenza B are highest for children and decrease with age [24,25]. However, the major difference that was seen for the season 2017/18 was the higher proportion of adult patients. The age-subgroups themselves, being pediatric patients and adult patients, were not different in their clinical characteristics between 2017/18 and the two previous seasons. However, the adult population that was seen had a high proportion of comorbidities and risk factors that might have contributed to a

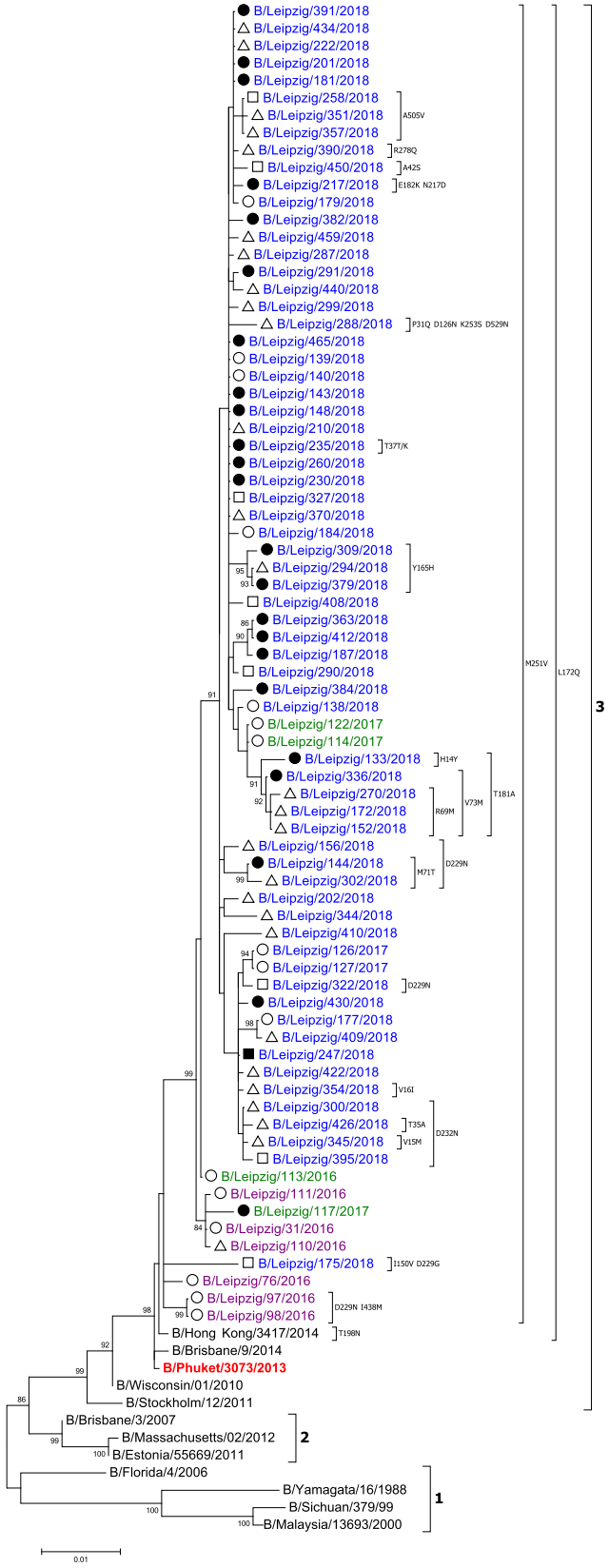

Fig. 3 (*continued*)

Table 3
Amino acid changes of the Victoria- and Yamagata-strains with regard to the respective vaccine strain.

		HA0																																					
		HA1																												HA2									
Amino Acid Position		14	15	16	31	35	37	42	69	71	73	80	117	126	127	129	150	165	172	181	182	197	217	229	232	251	253	258	278	313	346	408	417	418	438	505	525	529	
Antigenic Region													A	A	A	A	B	C		A		D																	
B/Brisbane/60/2008		H	V	V	P	T	T	A	R	K	T	R	I	N	A	N	N	K	P	C	T	N	A	G	P	M	Q	T	G	E	E	K	M	D	T	D	N	L	B/Victoria
B/Leipzig/59/2016	△	·	·	·	·	·	·	·	·	·	·	·	V	·	·	D	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	
B/Leipzig/66/2016	○	·	·	·	·	·	·	·	·	·	·	·	V	·	·	D	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	
B/Leipzig/25/2016	□	·	·	·	·	·	·	·	·	·	·	·	V	·	·	D	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	
B/Leipzig/70/2016	○	·	·	·	·	·	·	·	·	·	·	·	V	·	·	D	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	I	·	·	·	·	·	
B/Leipzig/125/2017	○	·	·	·	·	·	·	·	·	·	·	G	V	·	·	D	·	·	·	·	·	·	·	·	·	·	·	·	·	K	·	·	·	N	·	·	·	·	
B/Leipzig/83/2016	△	·	·	·	·	·	·	·	·	·	·	·	V	·	·	D	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	
B/Leipzig/40/2016	○	·	·	·	·	·	·	·	·	·	·	·	V	·	·	D	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	
B/Leipzig/90/2016	●	·	·	·	·	·	·	·	·	·	·	·	V	·	·	D	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	
B/Leipzig/44/2016	●	·	·	·	·	·	·	·	·	·	·	·	V	·	·	D	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	S	·	
B/Leipzig/96/2016	○	·	·	·	·	·	·	·	·	·	·	·	V	·	·	D	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	
B/Leipzig/22/2016	●	·	·	·	·	·	·	·	·	·	·	·	V	·	·	D	·	·	·	·	·	D	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	
B/Leipzig/112/2016	○	·	·	·	·	·	·	·	·	·	·	·	·	·	·	D	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	·	·	·	·	·	
B/Leipzig/86/2016	△	·	·	·	·	·	·	·	·	·	·	·	·	·	·	D	·	·	·	·	·	·	·	·	·	·	·	I	·	·	·	·	·	·	·	·	·	·	
B/Leipzig/46/2016	△	·	·	·	·	·	·	·	·	·	·	·	·	·	·	D	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	R	·	·	·	·	·	·	
B/Leipzig/33/2016	△	·	·	·	·	·	·	·	·	·	·	·	·	·	T	D	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	
B/Leipzig/56/2018	○	·	·	·	·	·	·	·	·	·	·	·	·	·	·	D	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	
B/Phuket/3073/2013		H	V	V	P	T	T	A	R	M	V	K	I	D	A	K	I	Y	L	T	E	K	N	D	D	M	K	G	R	H	R	K	D	E	I	A	D	D	B/Yamagata
48 strains	*	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	Q	·	·	·	·	·	·	V	·	·	·	·	·	·	·	·	·	·	·	·	
B/Leipzig/258/2018	□	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	Q	·	·	·	·	·	·	V	·	·	·	·	·	·	·	·	·	V	·	·	
B/Leipzig/351/2018	△	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	Q	·	·	·	·	·	·	V	·	·	·	·	·	·	·	·	·	V	·	·	
B/Leipzig/357/2018	△	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	Q	·	·	·	·	·	·	V	·	·	·	·	·	·	·	·	·	A/V	·	·	
B/Leipzig/390/2018	△	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	Q	·	·	·	·	·	·	V	·	·	Q	·	·	·	·	·	·	·	·	·	
B/Leipzig/450/2018	□	·	·	·	·	·	·	S	·	·	·	·	·	·	·	·	·	·	Q	·	·	·	·	·	·	V	·	·	·	·	·	·	·	·	·	·	·	·	
B/Leipzig/217/2018	●	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	Q	·	K	·	D	·	·	V	·	·	·	·	·	·	·	·	·	·	·	·	
B/Leipzig/288/2018	△	·	·	·	Q	·	·	·	·	·	·	·	·	N	·	·	·	·	Q	·	·	·	·	·	·	V	S	·	·	·	·	·	·	·	·	·	·	N	
B/Leipzig/235/2018	●	·	·	·	·	·	T/K	·	·	·	·	·	·	·	·	·	·	·	Q	·	·	·	·	·	·	V	·	·	·	·	·	·	·	·	·	·	·	·	
B/Leipzig/309/2018	●	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	H	Q	·	·	·	·	·	·	V	·	·	·	·	·	·	·	·	·	·	·	·	
B/Leipzig/294/2018	△	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	H	Q	·	·	·	·	·	·	V	·	·	·	·	·	·	·	·	·	·	·	·	
B/Leipzig/379/2018	●	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	H	Q	·	·	·	·	·	·	V	·	·	·	·	·	·	·	·	·	·	·	·	
B/Leipzig/133/2017	●	Y	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	Q	A	·	·	·	·	·	V	·	·	·	·	·	·	·	·	·	·	·	·	
B/Leipzig/336/2018	●	·	·	·	·	·	·	·	·	·	M	·	·	·	·	·	·	·	Q	A	·	·	·	·	·	V	·	·	·	·	·	·	·	·	·	·	·	·	
B/Leipzig/152/2018	△	·	·	·	·	·	·	·	M	·	M	·	·	·	·	·	·	·	Q	A	·	·	·	·	·	V	·	·	·	·	·	·	·	·	·	·	·	·	
B/Leipzig/172/2018	△	·	·	·	·	·	·	·	M	·	M	·	·	·	·	·	·	·	Q	A	·	·	·	·	·	V	·	·	·	·	·	·	·	·	·	·	·	·	
B/Leipzig/270/2018	△	·	·	·	·	·	·	·	M	·	M	·	·	·	·	·	·	·	Q	A	·	·	·	·	·	V	·	·	·	·	·	·	·	·	·	·	·	·	
B/Leipzig/156/2018	△	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	Q	·	·	·	·	N	·	V	·	·	·	·	·	·	·	·	·	·	·	·	
B/Leipzig/144/2018	●	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	Q	·	·	·	·	N	·	V	·	·	·	·	·	·	·	·	·	·	·	·	
B/Leipzig/302/2018	△	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	Q	·	·	·	·	N	·	V	·	·	·	·	·	·	·	·	·	·	·	·	
B/Leipzig/322/2018	□	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	Q	·	·	·	·	N	·	V	·	·	·	·	·	·	·	·	·	·	·	·	
B/Leipzig/354/2018	△	·		I	·	·	·	·	·	·	·	·	·	·	·	·	·	·	Q	·	·	·	·	·	·	V	·	·	·	·	·	·	·	·	·	·	·	·	
B/Leipzig/300/2018	△	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	Q	·	·	·	·	·	N	V	·	·	·	·	·	·	·	·	·	·	·	·	
B/Leipzig/426/2018	△	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·	Q	·	·	·	·	·	N	V	·	·	·	·	·	·	·	·	·	·	·	·	
B/Leipzig/345/2018	△	·	M	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	Q	·	·	·	·	·	N	V	·	·	·	·	·	·	·	·	·	·	·	·	
B/Leipzig/395/2018	□	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	Q	·	·	·	·	·	N	V	·	·	·	·	·	·	·	·	·	·	·	·	
B/Leipzig/175/2018	□	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	V	·	Q	·	·	·	·	G	·	V	·	·	·	·	·	·	·	·	·	·	·	·	
B/Leipzig/97/2016	○	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	Q	·	·	·	·	N	·	V	·	·	·	·	·	·	·	·	M	·	·	·	
B/Leipzig/98/2016	○	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	Q	·	·	·	·	N	·	V	·	·	·	·	·	·	·	·	M	·	·	·	

Amino acids are numbered with regard to the HA0 protein, and HA1 as well as HA2 subunits are indicated. The vaccine strains to which the isolates were compared are given in red for each lineage. Isolates from this study are labeled as vaccinated with trivalent vaccine (triangle), vaccinated with tetravalent vaccine (square), randomly selected case without vaccination history (circle), fatal cases (black filled symbols). Symbols represent the main antigenic sites, 120-loop (A; amino acids 116–137, 38, 56, 75, 177, 179–181), 150-loop (B; amino acids 141–150), 160-loop (C; amino acids 162–167) and 190-helix (D; amino acids 194–202). *For the Yamagata-lineage, 48 strains, that were identical at amino acid level, are depicted as a pool and contain patients with the following characteristics: 15x○; 15x●; 3x□, 1x■, 14x△.

severe outcome of the influenza disease, as it is known for example for cardiac insufficiency [26], additionally to age itself [2,27,28]. A shift towards the adult population thus increases the impact on the public health system and may explain the severity of this season with a high number of excess consultations. An interpretation of the presented clinical data, however, needs to be done with caution as there may be a bias towards severe disease courses in a hospital setting. A fatality rate of 11.2% of the adult population of this study can thus not be transferred to the general population.

A third potential factor may be the circulating influenza type. The Yamagata-lineage of influenza B was predominantly found in the season of 2017/18. In contrast to the Victoria lineage, which has a faster evolution with nearly each season representing a phylogenetic bottleneck, the biology of the Yamagata lineage is different. In long term studies of the HA of the Yamagata lineage a long co-circulation of Yamagata variants was observed [3,13,29]. In accordance with these observations the analyzed strains of this study were phylogenetically closely related. Furthermore, the detected amino acid changes were scattered throughout the HA and to the best of our knowledge no substitution is known for increased virus pathogenicity.

Since 2000, changes in the antigenic sites are dominantly located in the 120-loop and 190-helix regions, indicating main targets for immune pressure. With regard to the vaccine strain B/Phuket/3073/2013 the main antigenic regions were only affected in 9 strains and occurred mainly in the 120-loop region, followed by the 160-loop region for the analyzed Yamagata strains. In contrast, all analyzed Victoria strains had at least one amino acid change in the 120-loop region with regard to the vaccine strain B/Brisbane/60/2008. It is therefore surprising that the Yamagata-lineage that circulated in the population for three consecutive seasons was able to cause such a major epidemic.

A reason for this might be the interplay between the circulating influenza strains causing infections and the choice of the vaccine components or type of vaccine. In the season of 2015/16 Influenza A/H1N1 pdm09 and the Victoria-lineage of influenza B caused the majority of influenza infections. In 2016/17 influenza A/H3N2 was the main cause of flu disease. When looking at the influenza B component of the broadly used trivalent influenza vaccine there was a switch from B/Phuket/3073/2013-like (Yamagata-lineage) to B/Brisbane/60/2008-like (Victoria-lineage) in the season 2016/17, effectively causing a mismatch in each of the three studied seasons. In 2017/18 the only strain that was not immunologically covered by recent epidemics or the current vaccine composition was the influenza B Yamagata-lineage. An increased proportion of non-immune individuals in the adult population thus may have contributed to the observed shift in the age distribution of the infected patients.

Cross-reactive antibodies can be isolated from vaccinated individuals [30,31] but the contribution to the immunity against influenza B is not well defined. Some degree of lineage cross-protection has been reported upon vaccination with trivalent influenza virus vaccines. However, it seems to be limited to certain age groups and the vaccine efficacy is consistently superior for influenza B strains belonging to the vaccine lineage [32,33,16]. The capability of an individual to generate a broad antibody response against epitopes that include regions conserved between the two lineages, e.g. in the HA stalk, seems to be of major importance. Thus, the factors influencing lineage cross-protection are complex and not easy to predict as also the immune memory may have a substantial impact on the targeted epitopes [34,35].

In 76% of the patients with a vaccination history in this study the trivalent vaccine, with an expected moderate level of vaccine efficiency [7], was used. The majority of the vaccinated patients were adults. However, a major improvement to influenza disease burden may only be accomplished by extending vaccine recommendations to younger age groups that are immunologically more naïve and thus more susceptible to infections [36,25]. WHO recommendations for the use of tetravalent vaccines that contain two influenza B strains (Yamagata and Victoria) in addition to the two influenza A strains (H1N1 and H3N2) should be implemented in national vaccination guidelines as it was done in Germany as a result of the severity of the 2017/18 influenza season.

Author contribution

Study design: UGL
Data collection: MH, DM, SB
Data analysis: MH
Data interpretation: MH, UGL
Writing: MH, CP, MM, EB, UGL.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.08.027.

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