RESPIRATORY SYNCYTIAL VIRUS ACUTE RESPIRATORY INFECTIONS IN ≥65-YEAR-OLD ADULTS IN LONG-TERM CARE FACILITIES IN THE CZECH REPUBLIC

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SUMMARY

Objectives: Due to immunosenescence and presence of comorbidities, respiratory syncytial virus (RSV) disease burden is a major health concern in older adults, which is expected to increase with the life expectancy rise. Data on RSV burden are scarce in older adults residing in long-term care facilities, a vulnerable population living in crowded settings. Therefore, two independent prospective studies were conducted during the 2003–2004 and 2004–2005 RSV seasons to assess RSV acute respiratory illnesses (ARIs) and lower respiratory tract infections (LRTIs) in ≥65-year-old adults residing in long-term care facilities in the Czech Republic.

Methods: RSV ARI episodes were confirmed by polymerase chain reaction in nasal swabs collected within 3 days of symptoms onset. The mortality and morbidity of RSV-confirmed ARIs, as well as the risk factors associated with RSV-confirmed ARIs were evaluated.

Results: Among 1,251 participants in the 2003–2004 season (ARI surveillance between October and March), there were no RSV-positive cases in 255 ARI and 105 LRTI episodes. Among 1,280 participants in the 2004–2005 season (ARI surveillance between October and April), there were 39 and 26 RSV-positive cases in 335 ARI and 217 LRTI episodes, respectively, and RSV-positive ARI and LRTI episode incidence rates were 45.82 and 30.40 per 1,000 person-years. Among 290 RSV-negative and 39 RSV-positive ARI cases in the 2004–2005 season, 15 and 4 hospitalizations, 188 and 26 LRTIs, and 11 and 3 deaths were reported. Risk factors associated with RSV-positive ARI were female gender (odds ratio: 4.98), chronic heart failure class II (odds ratio: 2.31) and diabetes requiring insulin treatment (odds ratio: 9.82).

Conclusions: These studies showed that RSV was an important cause of ARI in older adults living in long-term care facilities in the 2004–2005 season, with fluctuating yearly incidences.

Key words: acute respiratory illness, clinical symptoms, Czech Republic, incidence, long-term care facilities, lower respiratory tract infection, older adults, respiratory syncytial virus, risk factors

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INTRODUCTION

Due to immunosenescence and presence of comorbidities, respiratory syncytial virus (RSV) infections are a major health concern in older adults, which is expected to increase with the steady life expectancy rise (1–6). In this population, RSV-associated disease rates are comparable to those of influenza (5, 7). RSV infections in hospitalized ≥60-year-old adults result in comparable or even more severe morbidity and mortality than influenza infections, and are associated with longer hospitalizations and greater odds of pulmonary complications, intensive care unit admissions and 1-year mortality (3).

Several vaccines intended to protect older adults against RSV are in development, but none is currently available on the market (8). In this context, updated information on RSV burden is warranted to develop effective immunization strategies and evaluate their impact (9). Incidence rates of RSV infections are difficult to estimate for various reasons. First, RSV infections are not assessed in routine clinical practice and are underdiagnosed. Second, clinical symptoms of RSV-associated acute respiratory illnesses (ARIs) overlap with those of other prevalent viral respiratory diseases (10, 11). Third, there is a lack of consensus on the ARI case definition (10). In older adults, the estimation of RSV-associated ARI incidence rates is further complicated by the low viral loads of RSV infections and the relatively poor sensitivity of diagnosis methods (10, 11), even if these have improved markedly in the last decades with the development of molecular techniques, e.g., polymerase chain reaction (PCR) (12).

Besides the facts that data on RSV burden are scarce and there is a lack of RSV surveillance data in older adults (9, 11), most epidemiological studies in this population were conducted in the community or in hospitalized patients, and compared proportions of infections caused by RSV and other respiratory pathogens (2, 4, 5, 13). Few studies estimated the incidence rates and impact of RSV infections in long-term care facilities (LTCFs) residents, a vulnerable population living in crowded institutional settings (6, 14–16). We report here the results of two independent prospective cohort studies conducted during two consecutive RSV seasons to estimate RSV-associated ARI incidence rates and potential risk factors, and the proportion of complications and hospitalizations in older adults living in LTCFs in the Czech Republic.

MATERIALS AND METHODS

Study Design

Two prospective, descriptive, multicentre studies were conducted in two different cohorts of older adults living in 16 LTCFs in the areas of Hradec Králové and Pardubice in the Czech Republic between 1 August 2003 and 31 May 2004 (season 1), and between 1 August 2004 and 20 May 2005 (season 2). ARIs surveillance took place between October 2003 and March 2004 for season 1, and between October 2004 and April 2005 for season 2. LTCFs were defined as facilities with ≥100 beds, where nursing and personal care services are provided.

Study Objectives

The primary objectives of both studies were to evaluate incidence rates of RSV-confirmed ARIs and lower respiratory tract infections (LRTIs), including pneumonia, in older adults living in LTCFs. RSV ARIs and LRTIs were confirmed by real-time reverse transcriptase PCR (RT-qPCR).

Secondary objectives included the evaluation of the mortality and morbidity of RSV-confirmed ARIs, risk factors associated with RSV-confirmed ARIs, incidence rates of influenza infections, all-cause pneumonia and parainfluenza infections in the study population, and the specificity, sensitivity and concordance of the RT-qPCR methodology compared with serology for RSV detection. The monthly distribution of ARI and LRTI episodes was evaluated by RSV and influenza status in post-hoc analyses.

Study Population

Eligible participants were residents of LTCFs within two months (August–September) prior to the RSV season, who were ≥65 years old at enrolment, were expected to reside in a LTCF for at least the following 7 months, were able to communicate, and had provided informed consent. All residents meeting inclusion criteria and without exclusion criteria were selected.

Intramuscular influenza vaccination is considered standard medical care for older adults residing in LTCFs. Trivalent inactivated vaccine (Fluarix, GSK) was offered to all enrolled participants during the first two weeks of October. Participants who could not receive influenza vaccine due to medical reasons were eligible; however, those declining its administration were excluded. Other exclusion criteria included confirmed senile dementia, Alzheimer disease or other chronic psychiatric pathologies.

Case Definitions

Patients with ARI were defined as patients requiring medical attention and presenting ≥4 of the following signs/symptoms: nasal congestion, sore throat, cough, sputum, dyspnoea, rhinorrhoea, wheezing, rales, rhonchi, and fever (axillary temperature ≥38.0 °C in season 1 and ≥37.5 °C in season 2).

LRTI was defined as diagnosis of bronchitis, bronchopneumonia or pneumonia. Pneumonia and bronchopneumonia were confirmed by X-ray. Symptoms/signs suggesting clinical suspicion of pneumonia were cough (acute or aggravating), sputum production, dyspnoea, auscultation findings (moist rales or locally diminished breathing sounds), fever, chest or abdomen pain when breathing, and acute deterioration of general condition. In case of pneumonia suspicion, X-ray and collection of sputum for culture were required for confirmation.

Data Collection

Baseline information was collected at enrolment, including demographic characteristics, pneumococcal vaccination status within the last 3 years, date of entry in the LTCF, number of roommates, immunocompetence status, and whether the participant was receiving medical care for cardiac diseases, chronic obstructive pulmonary disease (COPD), diabetes, asthma, or cancer.

Pre-season blood samples and nasal swabs were taken at enrolment. Participants were monitored for ARI signs/symptoms during the RSV season. For each ARI case, acute samples (blood and nasal swab at onset of illness) and a convalescent sample (blood only, 28 days after onset of illness) were taken. ARI episodes were followed up regularly for 4 weeks until resolution of symptoms.

For RSV-associated mortality and morbidity evaluation, the following clinical features were considered: clinical syndromes (upper respiratory tract infection, LRTI, pneumonia and bronchopneumonia), interventions (hospitalization for RSV-positive ARI, medical attendance, medication – antibiotics) and outcomes (resolution of symptoms with or without sequelae, aggravation of heart failure or COPD, duration of ARI episode, duration of hospitalization for RSV-positive ARI and death due to RSV-positive ARI).

Laboratory Testing

Collected samples (nasal swabs and blood samples) were shipped on dry ice according to the same procedures in both seasons. Nasal swabs were stored at −20 °C and were tested for RSV, influenza and parainfluenza virus by RT-qPCR. The validated sensitivity of assays was determined based on viral plaque forming unit (pfu) for the different viruses (3 pfu/ml of sample for RSV-A and -B; 30 pfu/ml of sample for influenza A and B; 100 pfu/ml of sample for parainfluenza).

For each blood sample, RSV fusion glycoprotein levels were determined using an in-house enzyme-linked immunosorbent assay (ELISA). Serological diagnosis of RSV infection was defined as an ≥4-fold rise in antibody titres between acute and convalescent samples. Laboratory assays were performed at GSK

(Rixensart, Belgium) and Neomed Laboratories Inc. (Montreal, Canada).

Statistical Analyses

In each study, the planned sample size was 1,250 participants, in whom 350 ARI cases were anticipated to occur. Based on surveillance data, approximately 2% were expected to be RSV-positive ARIs. The total enrolled cohort included all enrolled participants with available data. The according-to-protocol (ATP) cohort included all evaluable participants (i.e., meeting eligibility criteria, complying with protocol-defined procedures and with no elimination criteria during the study). The ATP-ARI cohort included all evaluable participants with ARI episodes.

Frequencies were calculated for categorical variables; mean, median, standard deviation (SD), and minimum and maximum for continuous data. Incidence rates were calculated with 95% confidence intervals (CIs) using exact Poisson confidence limits. The follow-up period was calculated from enrolment until either the first RSV-positive ARI episode or the last follow-up.

Associations between RSV-positive ARIs and possible risk factors were assessed using Fisher's exact test or chi-square test in univariate analyses. All risk factors were assessed using logistic regression analyses performed at the participant level. The final model was selected using a backward selection approach and included all risk factors with p-values <0.1. After model selection, a final multivariable logistic regression provided estimates and 95% CIs of the odds ratios (ORs). Both age group and gender were included in the logistic regression model to control for their potential confounding effects. All available results were included in the regression analyses, without any imputation for missing data.

RSV diagnosis agreement between RT-qPCR and ELISA was evaluated using Kappa statistics and the McNemar test. The sensitivity, specificity, concordance, and positive and negative predictive values were calculated with corresponding 95% CIs.

Statistical analyses were performed using the SAS™ software version 9.2 Drug Development (SDD) web platform.

A summary contextualizing the results, the potential clinical research relevance and the impact of our study is described in the Plain Language Summary (Fig. 1).

RESULTS

Study Participants

In the first study (season 1), 1,251 participants were included in the total enrolled cohort, 1,249 in the ATP cohort, and 244 (in whom 255 ARI episodes were reported) in the ATP-ARI cohort (Fig. 2). In the second study (season 2), 1,280 participants were included in the total enrolled cohort, 1,277 in the ATP cohort, and 294 (in whom 335 ARI episodes were reported) in the ATP-ARI cohort (Fig. 2).

Populations were similar in the two seasons, with approximately 70% of participants enrolled in both studies. Baseline characteristics of all participants and of those included in the ATP-ARI cohorts are presented in Table 1. Most participants (99.3% in season 1 and 99.5% in season 2) were vaccinated against influenza.

Incidence Rates of RSV, Influenza and Parainfluenza Episodes

None of the 255 ARI episodes, of which 105 were LRTIs, were RSV-positive during season 1 (Table 2). During season 2, 335 ARI episodes (39 RSV-positive, 290 RSV-negative and 6 unknown), of which 217 were LRTIs (26 RSV-positive, 188 RSV-negative and 3 unknown), were detected. Incidence rates of RSV-positive ARIs and LRTIs were 45.82 (95% CI: 32.59–62.64) and 30.40 (95% CI: 19.86–44.54) per 1,000 person-years. While no ARI surveillance was conducted in April 2004 (season 1), the surveillance period included April in season 2. This difference in duration had likely no impact because only 19 ARI episodes, which were all RSV-negative, were reported in April 2005. During season 1 and season 2, 7 and 61 influenza episodes, and 20 and 16 parainfluenza episodes were reported, respectively. The incidence rate of influenza episodes was substantially higher in season 2: 71.25 (95% CI: 54.50–91.53) per 1,000 person-years than in season 1: 9.54 (95% CI: 3.84–19.66) per 1,000 person-years (Table 2). The incidence rate of parainfluenza episodes was 27.62 (95% CI: 16.87–42.65) per 1,000 person-years in season 1 and 18.59 (95% CI: 10.62–30.18) per 1,000 person-years in season 2.

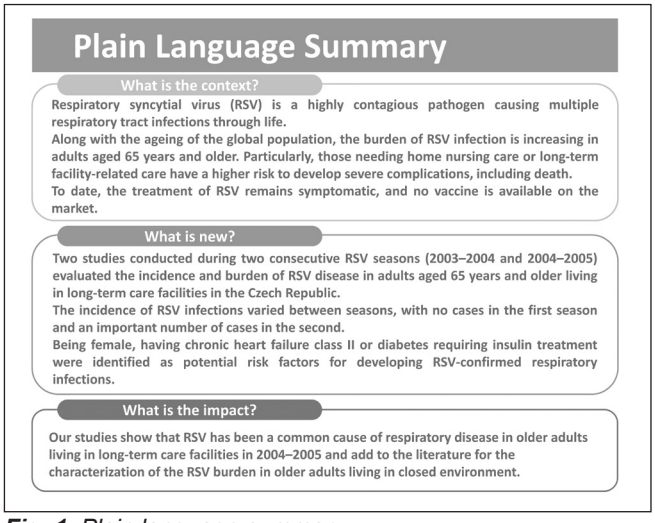

Fig. 1. *Plain language summary.*

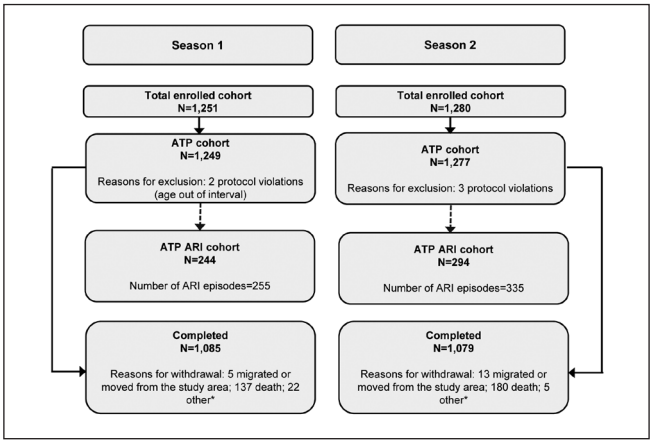

Fig. 2. *Flow of participants.*

Season 1 – 2003–2004 study; season 2 – 2004–2005 study; N – number of participants in each category; ATP – according to protocol; ARI – acute respiratory illness. *Other – participants not vaccinated against influenza due to medical reasons.

Table 1. *Demographic characteristics (ATP and ATP-ARI cohorts)*

Characteristics	Season 1		Season 2	
	All (N = 1,249)	ARI-positive (N' = 255)	All (N = 1,277)	ARI-positive (N' = 335)
Female gender, n (%)	898 (71.9)	188 (73.7)	914 (71.6)	237 (70.7)
Age (years)[a]				
≥ 75 years of age, n (%)	950 (76.1)	203 (79.6)	993 (77.8)	275 (82.1)
Mean (SD)	79.94 (7.42)	80.46 (7.04)	80.43 (7.33)	81.20 (6.61)
Median (min–max)	80.0 (65–97)	81.0 (65–98)	81.0 (65–100)	82.0 (66–95)
Sharing room, n (%)	1,020 (81.7)	209 (82.0)	1,039 (81.4)	279 (83.3)
Number of roommates, n (%)				
0	229 (18.3)	46 (18.0)	238 (18.6)	56 (16.7)
1	687 (55.0)	158 (62.0)	632 (49.5)	173 (51.6)
2	247 (19.8)	42 (16.5)	289 (22.6)	82 (24.5)
3	72 (5.8)	9 (3.5)	90 (7.0)	17 (5.1)
> 3	14 (1.1)	–	28 (2.2)	7 (2.1)
TIV vaccination, n (%)	1,217 (99.3)[b]		1,245 (99.5)[b]	
Comorbidities, n (%)				
HTA	660 (52.8)	149 (58.4)	728 (57.0)	202 (60.3)
Chronic heart failure class II	372 (29.8)	83 (32.5)	465 (36.4)	136 (40.6)
Diabetes type II	427 (34.2)	90 (35.3)	433 (33.9)	108 (32.2)
Angina pectoris	288 (23.1)	68 (26.7)	318 (24.9)	102 (30.4)
COPD	170 (13.6)	53 (20.8)	183 (14.3)	64 (19.1)
Cancer	92 (7.4)	20 (7.8)	103 (8.1)	29 (8.7)
Chronic heart failure class III	88 (7.0)	17 (6.7)	83 (6.5)	21 (6.3)
Diabetes requiring insulin treatment	52 (4.2)	16 (6.3)	39 (3.1)	10 (3.0)
Immunocompromised	9 (0.7)	2 (0.8)	18 (1.4)	4 (1.2)
Asthma	19 (1.5)	5 (2.0)	13 (1.0)	6 (1.8)
Chronic heart failure class IV	7 (0.6)	2 (0.8)	3 (0.2)	0 (0.0)

ATP – according to protocol; ARI – acute respiratory infections; N – number of participants in ATP cohort; N' – number of ARI episodes for participants in ATP-ARI cohort; n (%) – number (percentage) of participants/episodes in a given category; SD – standard deviation; min–max – minimum–maximum; TIV – Trivalent inactivated influenza vaccination; HTA – hypertension arterial; COPD – chronic obstructive pulmonary disease.

[a]The reference date for calculation of age was different for the ATP cohort (age at enrolment) and for the ARI-positive cohort (age at onset of ARI).

[b]N = 1,225 for season 1 (2003–2004 study) (1,217 were vaccinated, 8 unvaccinated [health issues during the vaccination period] and no information on influenza vaccination status was available for the remaining participants) and N = 1,251 for season 2 (2004–2005 study) (1,245 were vaccinated, 6 unvaccinated [health issues during the vaccination period] and no information on influenza vaccination status was available for the remaining participants).

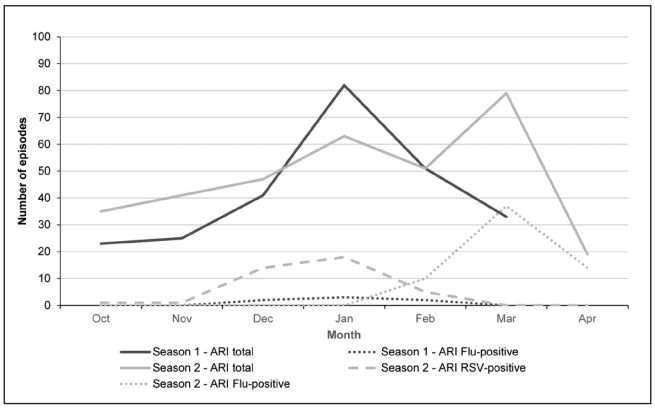

Fig. 3. *Monthly distribution of ARI, RSV-positive ARI and influenza-positive ARI episodes (ATP-ARI cohort).*

ARI – acute respiratory illness; RSV – respiratory syncytial virus; ATP – according to protocol; season 1 – 2003–2004 study; season 2 – 2004–2005 study.

Post-hoc analyses of the monthly distribution of ARI episodes showed a peak in January in season 1 and in March in season 2 (Fig. 3). In season 1, no RSV-positive ARIs and no peak of influenza episodes occurred. In season 2, a peak of RSV-positive ARI episodes was observed in January and a peak of influenza episodes in March. While no peak of LRTIs was observed in season 1, the monthly distribution of LRTIs was similar to that of ARIs in season 2 (data not shown).

RSV-Positive and -Negative ARI Episodes

The median duration of ARI episodes was 12 days (RSV-negative during season 1), 13 days (RSV-negative during season 2) and 12 days (RSV-positive during season 2) (Table 3). Among 255 RSV-negative ARIs in season 1, 15 (5.9%) hospitalizations, with a median duration of 20.5 days, and 11 (4.3%) deaths were

Table 2. *Incidence and number of RSV, influenza and parainfluenza episodes (ATP cohort)*

	Total number of episodes (number of patients)[a]	Number of RSV-positive episodes (%)[b]	Number of RSV-negative episodes (%)[b]	Number of episodes with an unknown RSV status (%)[b]	Incidence of positive infections per 1,000 person-years (95% CI)
Season 1					
RSV-ARI	255 (244)	0[c]	–	–	0.00 (0.00–5.02)
RSV-LRTI	105 (101)	0	–	–	0.00 (0.00–5.02)
Influenza	7 (7)	–	–	–	9.54 (3.84–19.66)
Parainfluenza	20 (20)	–	–	–	27.62 (16.87–42.65)
Season 2					
RSV-ARI	335 (294)	39 (11.64)	290 (86.57)	6 (1.79)[d]	45.82 (32.59–62.64)
RSV-LRTI	217 (191)	26 (11.98)	188 (86.64)	3 (1.38)	30.40 (19.86–44.54)
Influenza	61 (61)	–	–	–	71.25 (54.50−91.53)
Parainfluenza	16 (16)	–	–	–	18.59 (10.62−30.18)

RSV – respiratory syncytial virus; ATP – according to protocol; CI – confidence interval;season 1 – 2003–2004 study (ARI surveillance between October 2003 and March 2004); ARI – acute respiratory infections; LRTI – lower respiratory tract infections; season 2, 2004–2005 study (ARI surveillance between October 2004 and April 2005).
[a]Some patients had more than 1 episode.
[b]Percentage compared to the total number of ARI or LRTI episodes.
[c]In season 1, all ARI and LRTI episodes with available results were RSV-negative; one ARI episode had no results available.
[d]Swab sample not collected or laboratory results not available.

Table 3. *Description of clinical features for ARI episodes (ATP-ARI cohort)*

Clinical feature		Season 1	Season 2	
		RSV-negative ARI (N = 255)	RSV-negative ARI (N = 290)	RSV-positive ARI (N = 39)
Hospitalizations during the ARI follow-up period				
Number of hospitalizations, n (%)		15 (5.9)	15 (5.2)	4 (10.3)
Median duration in days (min–max)		20.5 (2.0–95.0)	15 (8.0–50.0)	18.5 (14.0–37.0)
Complications of ARI episodes, n (%)				
Aggravation of COPD		13 (5.1)	13 (4.5)	0 (0)
Aggravation of heart failure		8 (3.1)	4 (1.4)	1 (2.6)
LRTI	Bronchitis	88 (34.5)	162 (55.9)	24 (61.5)
	Bronchopneumonia	16 (6.3)	23 (7.9)	4 (10.3)
	Pneumonia	1 (0.4)	3 (1.0)	0 (0)
Median duration of ARI episode in days (min–max)		12 (2.0–34.0)	13 (3.0–36.0)	12 (5.0–32.0)
Resolution of symptoms (full recovery), n (%)		235 (92.2)	281 (96.9)	35 (89.7)
Mortality, n (%)		11 (4.3)	11 (3.8)	3 (7.7)

ARI – acute respiratory infections; ATP – according to protocol; season 1 – 2003–2004 study; season 2 – 2004–2005 study; RSV – respiratory syncytial virus; N – number of patients in a category; n (%) – number (percentage) of clinical features in a given category; min–max – minimum–maximum; COPD – chronic obstructive pulmonary disease; LRTI – lower respiratory tract infections.

reported (Table 3). Among 290 RSV-negative ARIs in season 2, 15 (5.2%) hospitalizations, with a median duration of 15 days, and 11 (3.8%) deaths were reported. Among 39 RSV-positive ARIs in season 2, 4 (10.3%) hospitalizations, with a median duration of 18.5 days, and 3 (7.7%) deaths were reported.

Of 255 RSV-negative ARIs in season 1, 88 (34.5%) were bronchitis cases, 16 (6.3%) bronchopneumonia cases and 1 (0.4%) a pneumonia case (Table 3). Among 290 RSV-negative ARIs in season 2, 162 (55.9%) were bronchitis cases, 23 (7.9%) bronchopneumonia cases and 3 (1.0%) pneumonia cases. Among 39 RSV-positive ARIs in season 2, 24 (61.5%) were bronchitis cases and 4 (10.3%) bronchopneumonia cases.

Among all RSV-negative and -positive ARI cases in both seasons, the most common clinical symptoms were cough (range: 95.9–97.4%), rhinorrhoea (89.7–94.9%) and nasal congestion (74.8–92.3%) (Table 4). Among 61 influenza-positive ARIs in season 2, the most common clinical symptoms were cough (98.4%), fever (88.5%) and rhinorrhoea (85.2%).

Among 39 RSV-positive ARIs in season 2, 28 (71.8%) were caused by RSV-A and 11 (28.2%) by RSV-B (Table 5). The most common clinical symptoms for ARI caused by RSV-A and RSV-B were similar: cough (100% and 90.9%), nasal congestion (96.4% and 81.8%) and rhinorrhoea (92.9% and 100%). Among 26 RSV-positive LRTIs, 19 (73.1%) were caused by RSV-A and 7 (26.9%) by RSV-B. Bronchitis was identified in 18 (64.3%) RSV-A-positive and 6 (54.5%) RSV-B-positive episodes, and bronchopneumonia in 2 (7.1%) RSV-A-positive and 2 (18.2%) RSV-B-positive episodes.

Table 4. *Description of clinical symptoms for ARI, either negative or positive for RSV, and influenza episodes (ATP-ARI cohort)*

Symptom/sign	Season 1		Season 2							
	RSV-negative ARI (N = 255)		RSV-negative ARI (N = 290)		RSV-positive ARI (N = 39)		Flu-negative ARI (N = 268)		Flu-positive ARI (N = 61)	
	n	%	n	%	n	%	n	%	n	%
Cough	247	96.9	278	95.9	38	97.4	256	95.5	60	98.4
Rhinorrhoea	236	92.5	260	89.7	37	94.9	245	91.4	52	85.2
Nasal congestion	232	91.0	217	74.8	36	92.3	207	77.2	46	75.4
Fever[a]	85	33.3	171	59	21	53.8	138	51.5	54	88.5
Sore throat	147	57.6	167	57.6	20	51.3	159	59.3	28	45.9
Sputum	96	37.6	129	44.5	15	38.5	113	42.2	31	50.8
Wheezing	63	24.7	89	30.7	13	33.3	79	29.5	23	37.7
Dyspnoea	67	26.3	110	37.9	12	30.8	90	33.6	32	52.5
Rales (crackles)	42	16.5	65	22.4	7	17.9	57	21.3	15	24.6
Ronchi	14	5.5	1	0.3	2	5.1	3	1.1	0	0.0

ARI – acute respiratory infections; ATP – according-to-protocol; season 1 – 2003–2004 study; season 2 – 2004–2005 study; RSV – respiratory syncytial virus; N – number of participants; n (%) – number (percentage) of symptoms in a given category.
[a]Fever is defined as an axillary temperature ≥ 37.5 °C.

Table 5. *Clinical symptoms for ARI episodes and clinical syndromes for LRTI and URTI episodes by RSV type in the 2004–2005 study (ATP-ARI cohort)*

	RSV-A (N = 28)		RSV-B (N = 11)	
	n	%	n	%
Clinical symptoms				
Cough	28	100.0	10	90.9
Rhinorrhoea	26	92.9	11	100.0
Nasal congestion	27	96.4	9	81.8
Fever[a]	13	46.4	8	72.7
Sore throat	14	50.0	6	54.5
Sputum	12	42.9	3	27.3
Wheezing	11	39.3	2	18.2
Dyspnoea	8	28.6	4	36.4
Rales (crackles)	6	21.4	1	9.1
Ronchi	1	3.6	1	9.1
Clinical syndromes				
Pneumonia	0	0.0	0	0.0
Bronchitis	18	64.3	6	54.5
Bronchopneumonia	2	7.1	2	18.2
URTI	17	60.7	9	81.8

ARI – acute respiratory infections; LRTI – lower respiratory tract infections; URTI – upper respiratory tract infections; RSV – respiratory syncytial virus; ATP – according to protocol; N – number of episodes in a category; n (%) – number (percentage) of clinical features in a given category.
[a]Fever is defined as an axillary temperature ≥ 37.5 °C.

Risk Factors for RSV-Positive ARI Episodes in Season 2

Among explored risk factors, being female (OR: 4.98, 95% CI: 1.62–15.33), having chronic heart failure class II (OR: 2.31, 95% CI: 1.13–4.73) and diabetes requiring insulin treatment (OR: 9.82, 95% CI: 2.20–43.90) were statistically significantly associated with RSV-positive ARIs (Table 6).

Comparison of RT-qPCR and ELISA for RSV diagnosis in Season 2

Of 39 participants diagnosed as RSV-positive by RT-qPCR, 36 had blood samples available at acute and convalescent stages. Of these, 25 had a 4-fold rise in antibody titres between both timepoints. Of 277 participants with ARIs, diagnosed as RSV-negative, 5 had a 4-fold rise in antibody titres between these timepoints

Table 6. *Estimated adjusted odds ratio for exploring risk factors associated with RSV-positive ARI participants in the 2004–2005 study (ATP-ARI cohort)*

Characteristics	n	OR	95% CI		p-value
			LL	UL	
Gender (female vs. male*)	35	4.98	1.62	15.33	0.0051
Chronic heart failure class II (yes vs. no*)	23	2.31	1.13	4.73	0.0223
Diabetes requiring insulin treatment (yes vs. no*)	5	9.82	2.20	43.90	0.0028

RSV – respiratory syncytial virus; ARI – acute respiratory infections; ATP – according to protocol; CI – confidence interval; n – number of participants with ARI RSV in the reference category used in the model; OR – odds ratio; LL – lower limit; UL – upper limit.
*reference category used in the model
Risk factors were assessed by logistic regression analysis using a backward selection (using a significance level of <0.1). Other characteristics (age group [<75 vs. ≥75 years of age], race, sharing room, number of roommates, chronic heart failure class III, chronic heart failure class IV, angina pectoris, hypertension arterial, chronic obstructive pulmonary disease, asthma, diabetes type II, cancer, immunocompromised participant, pneumococcal vaccination within the last 3 years) were analysed at univariable level, but were not statistically significant in the final model.

Table 7. *Clinical diagnosis of RSV based on RT-qPCR and serology results (anti-fusion glycoprotein antibody levels measured by ELISA) in the 2004–2005 study (ATP-ARI cohort)*

RT-qPCR confirmed RSV	Seroconversion[a]	No seroconversion	Total	Cohen's kappa coefficient (κ)[b]	McNemar p-value[c]
Positive	25	11	36	0.7293	0.1336
Negative	5	272	277		
Total	30	283	313		

RSV – respiratory syncytial virus; RT-qPCR – real-time reverse transcriptase polymerase chain reaction; ELISA – enzyme linked immunosorbent assay; ATP – according-to-protocol; ARI – acute respiratory infections.
[a]Seroconversion is defined as a 4-fold increase in antibody titres measured by ELISA between the onset and 28 days after the onset of illness.
[b]The magnitude of the Cohen's kappa coefficient (κ) represents the proportion of overall agreement greater than what would be expected by chance; k ranges from −1 (perfect disagreement) to 1 (perfect agreement), whereas a k of 0 indicates agreement equivalent to chance.
[c]Asymptotic McNemar p-value: McNemar's test is a statistical test used on paired nominal data. It is applied to 2 × 2 contingency tables with a dichotomous trait, with matched pairs of subjects, to determine whether the row and column marginal frequencies are equal and whether there is "marginal homogeneity".

(Table 7). Based on the correlation of RT-qPCR and serology results, a sensitivity of 69.4%, a specificity of 98.2% and a concordance of 94.9% were observed for RSV detection (Table 8).

DISCUSSION

These prospective studies conducted during 2 consecutive RSV seasons in ≥65-year-olds residing in LTCFs in the Czech Republic provide a relevant contribution to the characterization of the RSV burden in this important setting. In the Czech Republic, the approximate 100,000 adults residing in LTCFs constitute a fragile and vulnerable population (17). During season 1 (2003–2004), there were no cases of RSV-positive ARIs or LRTIs. In contrast, incidence rates of 45.82 and 30.40 per 1,000 person-years for RSV-positive ARIs and LRTIs were observed during season 2 (2004–2005). While this difference between the two consecutive RSV seasons cannot be explained by heterogeneities in study design (e.g., nasal swab methodology, timepoints for sampling, sample storage and assays), it may be partially explained by some methodological discrepancies. The longer surveillance period in season 2 had probably only a limited impact since no RSV-positive ARIs were detected in the additional month in 2005. In contrast, the decrease of the required fever threshold in season 2 (axillary temperature ≥37.5 °C) compared with season 1 (axillary temperature ≥38.0 °C) may have had an impact on the number of ARI cases captured as high levels of fever may not be as frequent in older adults. While 33.3% of ARI cases were reported with fever ≥38.0 °C in season 1, there was a higher proportion of ARI cases (58.8%) with fever ≥37.5 °C in season 2. Of note, fever is not always included in ARI case definitions in the literature (4–6). Another potential explanation may be the difference in severity of ARI cases between seasons. Indeed, a combination of 4 signs and/or symptoms was required in our studies to meet the ARI case definition, whereas 1 or 2 symptoms are considered sufficient in many other studies. This may have led to an underestimation of milder RSV-positive ARI cases in season 1.

In our study, where most of the participants were vaccinated against influenza, the number of influenza cases was also substan-

Table 8. *Summary of concordance measures between RT-qPCR methodology and serology (4-fold increase in anti-fusion glycoprotein antibody titres measured by ELISA) used for RSV detection in the 2004–2005 study (ATP-ARI cohort)*

Measures	Proportions % (95% CI)
Sensitivity	69.4 (54.4–84.5)
Specificity	98.2 (96.6–99.8)
Concordance	94.9 (92.4–97.3)
Positive predictive value	83.3 (70.0–96.7)
Negative predictive value	96.1 (93.9–98.4)

RT-qPCR – real-time reverse transcriptase polymerase chain reaction; ELISA – enzyme linked immunosorbent assay; RSV – respiratory syncytial virus; ATP – according-to-protocol; ARI – acute respiratory infections; CI – confidence interval. sensitivity – true positive / (true positive + false negative) * 100; specificity – true negative / (true negative + false positive) * 100; concordance – (true positive + true negative) / total * 100; positive predictive value – true positive / (true positive + false positive) * 100; negative predictive value – true negative / (true negative + false negative) * 100.

tially higher in season 2 compared with season 1. This observation suggests that the discrepancies in RSV- and influenza-positive ARI incidence rates could partially be explained by differences in the study population characteristics, as more participants were immunocompromised, had chronic heart failure class II and had >1 roommate in season 2, even if approximately 70% of participants were the same in both studies. The seasonal variation in RSV and influenza incidence rates may also be an explanation. A lower exposure to RSV and influenza during the 2003–2004 season (season 1) compared with other seasons was previously reported in the Czech Republic (18, 19). In our study, the monthly distribution of ARI episodes peaked in January during season 1 and in March during season 2. Since no RSV-positive ARI episodes and only a few influenza episodes were reported in season 1, most observed ARI cases were probably caused by other respiratory viruses (e.g., parainfluenza, rhinovirus, metapneumovirus, or others). In season 2, the peak of ARI episodes observed in March coincided with the peak of influenza infections and the peak of incidence of RSV-positive ARIs occurred earlier (January), which is in line with previous observations showing that influenza virus epidemics may occur later than RSV in most temperate sites (20). Data from the national influenza surveillance network of the Czech Republic was consistent with our findings with an overall low number of reported ARI cases with an early and weak peak during the 2003–2004 season, while a more prominent and later peak (February–March) occurred during the 2004–2005 season (21). The higher number of influenza cases observed late in season 2 could be due to a drift of circulating influenza subtypes and lineages, resulting in a lower vaccine efficacy later in the season (21). Among influenza cases, almost all were caused by influenza A in season 1, while around 25% of cases were caused by influenza B in season 2 (21). A waning immune response in our immunosenescent population may also explain the late influenza peak in season 2 since the vast majority of participants received the influenza vaccine in October (22). A higher incidence rate of influenza-like illness in 2004–2005 compared with other seasons was also observed in Northern Italy (23, 24). The nosocomial spread, which is facilitated in closed environments such as LTCFs, probably also contributed to the increased incidence rates observed in season 2, when exposure to influenza and RSV were higher compared with season 1 (6).

Differences in case definitions, study designs and populations as well as seasonal variability complexify direct comparisons of our results with previous reports. Nevertheless, in a study conducted during a RSV outbreak in a LTCF in the United States, 32% of older adults with respiratory infections were RSV-positive, compared to 0% and 11.64% in our study (14). In a second study, the prevalence of RSV in a non-epidemic setting was estimated by multiplex PCR at 5% among residents of LTCFs with ARI (15). In a recent systematic review on the burden of respiratory infections among older adults in LTCFs, RSV incidence proportions were reported to range from 1.1% to 10.8% in 4 studies, an incidence rate of 12.4 cases per 1,000 persons was reported in a fifth study conducted in non-outbreak settings, and incidence proportions of 12.9% and 13.5% were reported in 2 studies in outbreak settings (25). In another study, the annual RSV infection rate was 6.5% among residents of 33 LTCFs in the United States (26). Of note, the case definition for ARI used in our studies was stringent since participants had to require medical attention and to have ≥ 4 symptoms to be classified as ARI-positive, while less symptoms are often considered sufficient in other studies (4, 14–16). Since no nasal swab was collected and no RT-qPCR for confirmation of RSV infection was performed in participants not meeting the ARI case definition, a less restrictive case definition could have led to the detection of RSV-positive ARI cases in season 1 and more cases in season 2.

In our study, RSV-positive ARI cases tended to be slightly more severe than RSV-negative ARI cases in terms of hospitalization and mortality rates, and proportions of LRTIs. This observation is in line with a previous study comparing RSV and influenza infections in hospitalized older adults (3).

Among participants with ARI, risk factors associated with RSV-positive ARI episodes in our studies were female gender, chronic heart failure class II and diabetes requiring insulin treatment. The identification of female gender as a significant risk factor could partly be attributed to the higher number of women enrolled in both studies (71.9% and 71.6%); however, a plausible explanation remains unknown. The importance of RSV in people with underlying heart and lung conditions has been previously reported (5, 6, 27–30).

Finally, the results of our studies allowed us to compare 2 methods of detection of RSV infection in adults, namely the measurement of antibody titres by ELISA or RSV ribonucleic acid (RNA) by RT-qPCR. A sensitivity of 69.4% and a specificity of 98.2% were observed for RSV detection considering 4-fold increase in antibody titres measured by ELISA between acute and convalescent samples as compared to the detection of RSV RNA by RT-qPCR. The high concordance between both methods (94.9%) suggests that the quality of the nasal swabs was still acceptable after 15 years of storage. However, we observed that 5 RSV-negative participants by RT-qPCR were RSV-positive when a 4-fold increase in antibody titres was used for the diagnosis of RSV, which shows the usefulness of the serology in conjunction with RT-qPCR. Of note, the 4-fold increase in antibody titres was used as cut-off for seroconversion based on common practice and was previously used in RSV surveillance studies (6).

The main strengths of these studies included their similar design and methodology, the largely similar population, the detection of several key respiratory viruses, the high compliance and availability of samples taken from older patients with ARI, and the sampling timing within 3 days following symptoms onset. Limitations of these studies included the fact that they were carried out independently, even if approximately 70% of participants were the same, and the stringent case definition used for ARI, which may have led to an underestimation of the number of ARI cases and complicates the comparison with other studies. A further limitation is their delayed publication; clinical data were available, but immunological and detection assays had to be refined and further developed.

CONCLUSIONS

These studies have shown that RSV was an important cause of respiratory disease in older adults living in LTCFs in the 2004–2005 season, that the incidence of RSV-associated ARIs can fluctuate from season to season, and that the use of well-characterized epidemiological data together with serological tools

can significantly contribute to assess the overall level of infection in this population. Our results also highlight the importance of a common clinical definition of ARI to be able to compare incidences of RSV across studies.

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Conflict of Interests

JB reports receiving grant from the GlaxoSmithKline group of companies (GSK) for conduction of clinical trial outside the submitted work. RD, JMD, OG and JYP are employees of GSK. TLN, ARV and FS were employees of GSK at the time the study was performed. JMD, TLN and FS hold shares from GSK as part of their former or current employment. JMD is a designated inventor on patents owned by the GSK group of companies. FS is currently an employee of Janssen, Pharmaceutical Companies of Johnson & Johnson.

Trademark Statement

Fluarix is a trademark owned by or licensed to the GSK group of companies. SAS is a registered trademark of SAS Institute Inc.

Sponsorship

GlaxoSmithKline Biologicals SA was involved in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, and approval of the manuscript; and decision to submit the manuscript for publication. GlaxoSmithKline Biologicals SA does not veto ongoing publications or control the decision about what journal to submit to, with ultimate decision on the target made by the coauthors. GlaxoSmithKline Biologicals SA covered all costs associated with developing and publishing this article.

Adherence to Ethical Standards

The study was conducted according to Good Epidemiology Practice, the International Guidelines for Ethical Review of Epidemiological Studies, and local rules and regulations of the country. The protocol and other relevant documents were approved by an appropriate Institutional Review Board/Independent Ethics Committee (Ethics Committee at Medical Association, Ltd., Hradec Králové; EC decisions 200308 and 200403). Written informed consent was obtained from each participant or their legally authorized representative prior to the performance of any study-specific procedures.

Authors' Contributions:

JB – conceptualization, investigation, project administration, writing, review and editing; ARV – conceptualization, methodology, project administration, supervision, writing, review and editing;

RD – methodology, formal analysis, validation, writing, review and editing; TLAN – formal analysis, validation, writing, review and editing; OG – formal analysis, validation, writing, review and editing; JYP – conceptualization, methodology, supervision, writing, review and editing; FS – methodology, supervision, writing, review and editing; JMD – conceptualization, methodology, project administration, supervision, writing, review and editing. All authors had full access to the data and granted their final approval of the paper before submission.

Data Availability

For reasons of privacy protection for study participants, GSK offers access to data and materials via controlled access. Anonymized individual participant data from this study plus the annotated case report form, protocol, reporting and analysis plan, data set specifications, raw dataset, analysis-ready dataset, and clinical study report are available for research proposals approved by an independent review committee. Proposals should be submitted to www.clinicalstudydatarequest.com. A data access agreement will be required.

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