SUMMARY
Introduction/Objective Pertussis is a vaccine-preventable disease that causes a large number of cases and hospitalizations worldwide. The aim of this study was to determine predictors of hospitalization in cases of pertussis among children under 10 years of age in the South Bačka District of Vojvodina Province, Serbia.

Methods Data for this observational study were obtained from inpatient and outpatient healthcare facilities in the South Bačka District from January 1, 2013 to December 31, 2016. We evaluated predictors of hospitalization among the patients who fulfilled the criteria of case definitions of pertussis proposed by the Global Pertussis Initiative. Pertussis was confirmed by DNA polymerase chain reaction or ELISA serology tests.

Results Out of 122 laboratory-confirmed pertussis cases, 43 (35.2%) were hospitalized. Apnea and pneumonia were associated with hospitalization, and all six hospitalized patients aged 0–3 months had cyanosis. Apnea was a good predictor of hospitalization among children with any duration of cough ($p < 0.05$). Among children with a cough that lasted longer than 14 days, post-tussive emesis or pneumonia or contact with a person who had a prolonged cough were associated with hospitalization ($p = 0.035$, $p = 0.042$, and $p = 0.046$, respectively). There were fewer hospitalizations in properly vaccinated cases than in partly or non-vaccinated cases between two months and four years of age ($p < 0.008$).

Conclusions Among the pertussis cases under 10 years of age, apnea, pneumonia, and cyanosis were factors associated with hospitalization. Immunization against pertussis corresponding to age reduces the disease severity and hospitalizations in children from two months to four years of age.

Keywords: pertussis; hospitalization; surveillance; epidemiology

INTRODUCTION

Pertussis (whooping cough) as a vaccine-preventable disease is a prevalent cause of acute cough in both children and adults occurring in outpatient and inpatient health care facilities [1]. Despite high immunization coverage, pertussis is still present around the world [2–5]. In 2016, more than 139,000 pertussis cases were reported worldwide [6]. The majority (approximately 95%) of infections occurred in developing countries; with most deaths occurring in young infants who were either unvaccinated or incompletely vaccinated [3, 4, 5]. Even in countries with high vaccination coverage, pertussis causes a high number of cases and hospitalizations [2, 5]. A dramatic resurgence of pertussis worldwide, with large outbreaks and deaths mainly in infants, has drawn the attention of healthcare providers [5].

Protection against pertussis was achieved only after completed three-dose primary vaccination series at approximately six months of age [2, 3, 7]. According to the annual reports in the South Bačka District of Vojvodina Province, Serbia, between 2013 and 2016, the average immunization coverage of pertussis was 95% for the primary series (at two, four, and six months), and 90% for one booster dose (one year after the third dose of the vaccine) [8].

The main goal of this study was to determine predictors of hospitalization in cases of pertussis among children under 10 years of age in the South Bačka District.

METHODS

Study design

The design and methods of improved surveillance of pertussis have been described previously [9, 10]. Surveillance of pertussis from inpatient and outpatient healthcare facilities in the South Bačka District was conducted for four consecutive years, in the period from January 1, 2013 to December 31, 2016. We included all children under 10 years of age, regardless of the duration of cough. Additionally, when children fulfilled one or more criteria of pertussis proposed by the Global Pertussis Initiative (GPI), they were enrolled after admission to healthcare facilities for a period of one week [9]. During the study period, we included children who were hospitalized at the Department of Pulmonology of the Institute for Child and Youth Health Care of Vojvodina (an inpatient facility), and at 11 health centers (the primary healthcare level) of the South Bačka District.

Eligible were children under 10 years of age who met one or more criteria of clinical case definitions of pertussis proposed by the GPI for two age groups (0–3 months old, and four months to nine years old) (Table 1).
We excluded children who did not fulfil the GPI clinical case definitions of pertussis proposed for the aforementioned age groups. This research was conducted as a part of the daily clinical routine practice. The training of all included physicians and nurses was conducted before starting our research. Verbal informed consent was obtained from parents or guardians of children at the moment of swab taking in accordance with national regulations. All data about the children were anonymized and de-identified.

Participants

We obtained children's demographic and clinical data as well as the data about vaccination against pertussis in a structured questionnaire to parents or guardians. At the primary healthcare level, vaccination status was obtained from the participants' vaccination records. Vaccination status at inpatient facilities was determined from a parental report of the child's vaccination record, and therefore it was checked from vaccination records at the primary healthcare level. The child's physician determined clinical management, including hospitalization and laboratory procedures. Depending on the clinical course of the disease, all clinical and laboratory data were obtained at inpatient or outpatient healthcare facilities.

Posterior nasopharyngeal swabs and whole blood samples (single-serum) from the patients were collected by trained physicians and nurses at inpatient and outpatient medical facilities, as well as at the Institute of Public Health of Vojvodina, Novi Sad. All samples were analysed at the Centre for Microbiology of the Institute of Public Health of Vojvodina. According to the GPI case definitions of pertussis, the type of laboratory method (real-time polymerase chain reaction or serology tests) depends on the duration of cough and on the age of the suspected patient (Table 1) [9].

As we previously described in detail, nasopharyngeal specimens were defined as positive if *Bordetella pertussis* was detected by the real-time polymerase chain reaction [10]. Additionally, ELISA antibody test from whole blood samples was considered positive if cut-off values were above 100 IU/mL. Because of potentially false positive results, we excluded all participants between four months and nine years of age who had been vaccinated within one year before the collection of whole blood samples [9].

### Statistical analysis

We examined an association between potential predictors of hospitalization regarding certain signs/symptoms, sex, the duration of cough, residence, asthma, prescribed antibiotics, diagnostic methods, and vaccination status. In accordance with the vaccination status, we divided participants into two groups: 1) properly vaccinated (children who received the number of vaccine doses corresponding to their age), and 2) partly or non-vaccinated participants (partly vaccinated children were the ones who received some but not all vaccines, while the non-vaccinated patients were those who did not receive any dose of pertussis vaccine). For the analysis of the association between hospitalization and vaccination status, we excluded patients under two months of age because they were below the vaccination age. The two-tailed Fisher’s exact test or χ² were used for associations between categorical variables, with the Yate’s correction for continuity used for the analysis of dichotomous variables, and the Mann–Whitney U-test for continuous variables. We calculated the difference between the laboratory-confirmed pertussis in inpatient and outpatient healthcare facilities using univariate and multivariate logistic regression models by the odds ratio with 95% confidence interval regarding certain signs/symptoms.

The results were considered statistically significant when the p-value of all applied models was < 0.05. The data were analyzed using the IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA) and MedCalc for Windows, Version 12.3.0 (MedCalc Software, Mariakerke, Belgium).

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Table 1. Clinical case definitions of pertussis and diagnostic tests proposed by the Global Pertussis Initiative for patients under 10 years of age

| Age groups | 0–3 months | 4 months – 9 years |
|------------|------------|-------------------|
| Signs/symptoms/contact | Cough and coryza with no or minimal fever plus: | Paroxysmal cough with no or minimal fever plus: |
| | – whoop or – apnea or – post-tussive emesis or – cyanosis or one of the following: – seizure – pneumonia – close exposure to an adolescent or adult (usually a family member) with a prolonged afebrile cough illness | – whoop or – apnea or one of the following: – post-tussive emesis – seizure – worsening of symptoms at night – pneumonia – close exposure to an adolescent or adult (usually a family member) with a prolonged afebrile cough illness |
| Diagnostic method – cough illness in a person with no or minimal fever plus cough duration* | PCR for all children aged 0–3 months | PCR or serology (IgG-PT), if ≥ 1 year post-pertussis vaccination |

*Adapted from the Global Pertussis Initiative; for patients aged four months to nine years: PCR if cough duration is ≤ 3 weeks, and serology if cough duration is > 3 weeks
RESULTS

General characteristics of children with laboratory-confirmed pertussis

During 2013–2016, 122 laboratory-confirmed pertussis cases under 10 years of age were reported. Of these, 43 (35.2%) were inpatients and 79 (64.7%) were outpatients.

| Variable                      | Total cases | Hospitalized | Outpatients |
|-------------------------------|-------------|--------------|-------------|
|                              | No. / total | No. / total  | No. / total |
|                              | %           | %            | %           |
|                              |             |              |             |
| Sex                           |             |              |             |
| Female                        | 59/122      | 23/43        | 36/79       |
|                               | (48.4)      | (53.5)       | (45.6)      |
| Male                          | 63/122      | 20/43        | 43/79       |
|                               | (51.6)      | (46.5)       | (54.4)      |
| Age                           |             |              |             |
| 0–3 months                    | 8/122       | 6/43         | 2/79        |
|                               | (6.6)       | (14.0)       | (2.5)       |
| 4–12 months                   | 11/122      | 9/43         | 2/79        |
|                               | (9.0)       | (20.9)       | (2.5)       |
| 2–5 years                     | 22/122      | 7/43         | 15/79       |
|                               | (18.0)      | (16.3)       | (19.0)      |
| 6–9 years                     | 81/122      | 21/43        | 60/79       |
|                               | (66.4)      | (48.8)       | (76.0)      |
| Duration of cough in days     | 25.5 ± 17.3 | 26.5 ± 19.3  | 25.0 ± 16.1 |
|                              |             |              |             |
| Residence                     |             |              |             |
| Urban area                    | 87/122      | 29/43        | 58/79       |
|                               | (71.3)      | (67.4)       | (73.4)      |
| Rural area                    | 35/122      | 14/43        | 21/79       |
|                               | (28.7)      | (32.6)       | (26.6)      |
| Asthma or bronchitis or laryngitis |           |              |             |
| Yes                           | 34/122      | 15/43        | 19/79       |
|                               | (27.9)      | (34.9)       | (24.1)      |
| No                            | 88/122      | 28/43        | 60/79       |
|                               | (72.1)      | (65.1)       | (75.9)      |
| Diagnostic method             |             |              |             |
| PCR positive                  | 37/122      | 17/43        | 20/79       |
|                               | (30.3)      | (39.5)       | (25.3)      |
| Serology (IgG-PT) positive    | 85/122      | 26/43        | 59/79       |
|                               | (69.7)      | (60.5)       | (74.7)      |
| Vaccination statusa           |             |              |             |
| Properly vaccinated according to age | 100/116       | 28/38       | 72/78       |
|                               | (86.2)      | (73.7)       | (92.3)      |
| Partly vaccinated or non-vaccinated | 16/116       | 10/38       | 6/78        |
|                               | (13.8)      | (26.3)       | (7.7)       |
| Antibiotic treatment before sampling |           |              |             |
| Yes                           | 25/122      | 14/43        | 11/79       |
|                               | (20.5)      | (32.6)       | (13.9)      |
| No                            | 97/122      | 29/43        | 68/79       |
|                               | (79.5)      | (67.4)       | (86.1)      |

SD – standard deviation; PCR – polymerase chain reaction; IgG – immunoglobulin G; PT – pertussis toxin; χ² test; Two-tailed Fisher’s exact test; Mann–Whitney test; Only for patients aged two months to nine years; Values that differ significantly (p < 0.05) between hospitalized and outpatient laboratory-confirmed cases are marked in bold

Risk factors for hospitalization

To assess the effects of certain signs/symptoms of clinical case definitions of pertussis for the two age groups and the vaccination status of participants, we compared the results of pertussis-positive children who were hospitalized with those who were not (Table 3, 4, and 5).

Taking into account the required signs/symptoms (RSS) in children aged 0–3 months and four months to nine years (Table 1), Table 3 shows the signs/symptoms in hospitalized and outpatient cases. The most frequent clinical sign/symptom among inpatients and outpatients was whoop (58.1% and 48.1%, respectively). In patients four months to nine years of age, the prevalence of worsening of symptoms at night was 72.7% in outpatients and 67.6% among inpatients. All six hospitalized patients aged 0–3 months with laboratory-confirmed pertussis had cyanosis. According to univariate and multivariate logistic regression analysis, we revealed that RSS in combination with apnea or pneumonia was associated with hospitalization (p < 0.05). Although the combinations of RSS and whoop or post-tussive emesis were not significantly associated with hospitalization, the association of these variables increased after adjustment for the confounding effect of the vaccination status.

The RSS along with apnea were a good predictor of hospitalization among children with any duration of cough (p < 0.05). In children who had a cough for more than 14 days, the RSS combined with post-tussive emesis or pneumonia or with information of close exposure to a person with a prolonged cough were associated with hospitalization (p = 0.035, p = 0.042, and p = 0.046, respectively) (Table 4).

We analyzed the association between hospitalization and vaccination in properly vaccinated cases and among those who were partly or non-vaccinated against pertussis. There were 116 pertussis cases two months to nine years of age. Of these, 38 (32.8%) were hospitalized. There were fewer hospitalizations in properly vaccinated cases than in partly or non-vaccinated cases. There were 116 pertussis cases two months to nine years of age. Of these, 38 (32.8%) were hospitalized. There were fewer hospitalizations in properly vaccinated cases than in partly or non-vaccinated cases (p = 0.008). However, there was no significant difference between hospitalization in properly and partly or non-vaccinated children against pertussis in patients 5–9 years old (p = 0.570) (Table 5).

DISCUSSION

This is the first study to evaluate predictors of hospitalization among laboratory-confirmed pertussis cases in our country. Our findings provide a comprehensive view of...
The obvious high prevalence of hospitalized cases lies in the fact that the authors of the mentioned study included not only the primary cases, but also all secondary cases of pertussis (contacts with primary cases).

Our results show that RSS in combination with apnea increased the probability of hospitalization by about four times and the combination of RSS accompanied with pneumonia by more than 15 times. In addition, among patients aged 0–3 months, a cough and coryza with no or minimal fever, as the RSS, combined with cyanosis was a good predictor of hospitalization. Considering a lot of research conducted with heterogeneous inclusion/exclusion criteria in varying clinical settings, with different types of diagnostic pertussis tests, as well as various immunization schedules, multiple studies reported different results regarding predictors of pertussis hospitalizations. The results of the aforementioned study highlighted that whoop, apnea, and cyanosis were more frequent in hospitalized than in outpatient cases, and pneumonia was not associated with an increasing risk of hospitalization [7]. The limitation of the mentioned study was the duration of the study period (only two years).

The results of another study, which was conducted among children with pertussis in a hospital setting, showed that children who were readmitted had more cyanotic episodes per day, with a greater number of hospitalizations. We revealed that 35.2% of laboratory-confirmed cases showed that children who were readmitted had more cyanotic episodes per day, with a greater number of hospitalizations.

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Table 3. Predictive signs/symptoms and contact in hospitalized and outpatients under 10 years of age in the South Bačka District, Vojvodina, 2013–2016

| Signs/symptoms/contact | Cough duration of ≤ 14 days (n = 39) | Cough duration of > 14 days (n = 83) |
|------------------------|--------------------------------------|--------------------------------------|
|                        | Hospitalized No. / total No. (%)     | Outpatients No. / total No. (%)      | p            | Hospitalized No. / total No. (%)     | Outpatients No. / total No. (%)      | p            |
| Whoop                  | 8/15 (53.3)                           | 14/24 (58.3)                          | NS           | 17/28 (60.7)                           | 22/55 (40)                            | NS           |
| Apnea                  | 6/15 (40)                             | 1/24 (4.2)                            | 0.008        | 12/28 (42.9)                           | 8/55 (14.5)                           | 0.007        |
| Post-tussive emesis    | 7/15 (46.7)                           | 10/24 (41.7)                          | NS           | 17/28 (60.7)                           | 19/55 (34.5)                          | 0.035        |
| Pneumonia              | 5/15 (33.3)                           | 0 (-)                                 | NA           | 4/28 (14.3)                            | 1/55 (1.8)                            | 0.042        |
| Contact                | 6/15 (40)                             | 9/24 (37.5)                           | NS           | 10/28 (35.7)                           | 8/55 (14.5)                           | 0.046        |

NS – not significant; NA – not applicable; aTwo-tailed Fisher’s exact test; bOnly for patients aged 0–3 months; cClose exposure to an adolescent or adult (usually a family member) with a prolonged afebrile cough illness; Values that differ significantly (p < 0.05) between hospitalized and outpatient laboratory-confirmed cases are marked in bold.

Table 4. Predictive signs/symptoms and contact in hospitalized and outpatients under 10 years of age in accordance with the cough duration before sampling in the South Bačka District, Vojvodina, 2013–2016

| Signs/symptoms/contact | Hospitalized No. / total No. (%)     | Outpatients No. / total No. (%)      | p            |
|------------------------|--------------------------------------|--------------------------------------|--------------|
| Whoop                  | 8/15 (53.3)                           | 14/24 (58.3)                          | NS           |
| Apnea                  | 6/15 (40)                             | 1/24 (4.2)                            | 0.008        |
| Post-tussive emesis    | 7/15 (46.7)                           | 10/24 (41.7)                          | NS           |
| Pneumonia              | 5/15 (33.3)                           | 0 (-)                                 | NA           |
| Contact                | 6/15 (40)                             | 9/24 (37.5)                           | NS           |

Table 5. Association between vaccination and hospitalization for pertussis among children aged two months two years in the South Bačka District, Vojvodina, 2013–2016

| Age group | Vaccination status | Hospitalized No. / total No. (%) | Outpatients No. / total No. (%) | p        |
|-----------|--------------------|----------------------------------|---------------------------------|----------|
| 2 months – 4 years n = 26 | Properly vaccinated | 4/14 (28.6)                      | 10/12 (83.3)                    | 0.008    |
|           | Partly vaccinated or non-vaccinated | 10/14 (71.4)                      | 2/12 (16.7)                     |          |
| 5–9 years n = 90 | Properly vaccinated | 24/24 (100)                       | 62/66 (93.9)                    | 0.570    |
|           | Partly vaccinated or non-vaccinated | 0 (-)                             | 4/66 (6.1)                      |          |

*Two-tailed Fisher’s exact test.
days [11]. Furthermore, the results of a recently published meta-analysis indicated that apnea and cyanosis are helpful for detection of pertussis in infants younger than 12 months of age [1].

Many of the implemented case definitions of pertussis predicted the cough duration of ≥ 2 weeks for patients of all ages. Due to the implementation of the new GPI case definitions of pertussis, which predicted inclusion of patients under 10 years of age, regardless of the cough duration, we found that as many as 32% (39/122) of the total number of cases had a cough duration of less than 14 days [9]. Our results are very important if we know that early diagnosis of pertussis in infants allows targeted antibiotic therapy, which could reduce the severity of the disease, the duration of cough and could play an important role in reducing pertussis transmission to close contacts. It is noteworthy that both vaccination and early treatment strategies are equally important for improving outcomes [12–16].

Observing the vaccination status among children from two months to four years of age, we clearly demonstrated an increasing risk of hospitalization among partly or non-vaccinated children in comparison with those who were fully immunized against pertussis. Multiple studies have reported similar results [7, 15–19]. Similar to the results of our research, a study conducted among infants (aged < 12 months) found that properly vaccinated children were protected against hospitalization [20]. In addition, probably because only participants aged < 12 months were included, the authors of the stated study revealed that protection against hospitalization was the same after immunization with whole-cell or acellular pertussis vaccines [20].

Our study findings suggest that the risk of hospitalization was the same regardless of vaccine doses among children aged 5–9 years. We believe that the reasons for this lie in the fact that vaccine-induced immunity waned over time, which consequently led to a decrease of the protective role of vaccination regarding the hospitalization. According to the recently published review data, the estimated duration of protection obtained from the whole-cell pertussis vaccine is 5–14 years, and the one from the acellular vaccine is 4–7 years [21, 22]. One of the recently mentioned explanations for the resurgence of pertussis worldwide, both in schoolchildren and adolescents is connected with changes in the antigens in circulating *Bordetella pertussis* in comparison with the vaccine strains [23].

In regard to the signs/symptoms, the results of the study among fully immunized children with the median age of nine years and the median cough duration of 14 days showed that only 21% of the patients had paroxysmal cough, 13% had post-tussive emesis, 7% apnea, and 6% had classic whoop [24]. Results of another study which was conducted on hospitalized children from one month to 15 years of age with prolonged cough (duration ≥ 14 days) who were previously vaccinated with four doses of vaccine against pertussis, demonstrated that the prevalence of paroxysmal cough was 84.4%, but post-tussive emesis and whoop were rare (31.3% and 28.2%, respectively) [25]. The mentioned differences can be interpreted as the result of various inclusion criteria of the study population. In our research, there were 74% of properly immunized hospitalized children from two months to nine years of age. Probably because of the implementation of active surveillance combined with training of the staff included in our research and low vaccination coverage among hospitalized children, there were 58.1% patients with whoop, 55.8% with post-tussive emesis, and as many as 41.9% children with apnea.

Due to the quality and comparability of the results of our study, we are convinced that this research has the potential to be a standard model in the preparation of more comprehensive hospital surveillance among children with pertussis infection throughout the Republic of Serbia.

**CONCLUSION**

We revealed that apnea, pneumonia, and cyanosis were good predictors of hospitalization in pertussis cases. In addition, apnoea was a good predictor for hospitalization among children, regardless of the duration of cough. On the other hand, post-tussive emesis, pneumonia, and contact with a person with the prolonged afebrile cough illness were associated with hospitalization among children with the cough duration of > 14 days. Immunization against pertussis corresponding to age reduces the disease severity and hospitalization in children from two months to four years of age.

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Велики кашаљ код деце млађе од десет година

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САЖЕТАК
Увод/Циљ Велики кашаљ је вакцином спречива зараза болест која је узрок великих броја оболелих, а хоспитализованих широм света. Циљ рада је био да се одреде предиспонирајући фактори за хоспитализацију оболелих код млађих од десет година у Јужнобачком округу (Војводина). Методе рада Подаци за ову опсервациону студију добијени су од болничких и ванболничких здравствених установа Јужнобачког округа у периоду од 1. јануара 2013. до 31. децембра 2016. године. Предиспонирајући фактори за хоспитализацију оболелих проценети су на основу клиничких критеријума (Global pertussis initiative criteria) за дечију кашаљу. Закључак За деце млађе од десет година, апнеа, пнеумонија и животни стање постојања после кашаља, пневмонија и контакт са оболелом који је имао дуготрајани кашаљ (р = 0,035, р = 0,042 и р = 0,046). Потпуно имунизована деца узраста од два до четири године била су ређе хоспитализована у односу на непотпуно имунизоване и невакциниране деца истог узраста (р < 0,008).

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