SERBIAN ARCHIVES OF MEDICINE Est.1872

Address: 1 Kraljice Natalije Street, 11000 Belgrade, Serbia
☎ +381 11 4092 776, Fax: +381 11 3348 653
E-mail: office@srpskiarhiv.rs, Web address: www.srpskiarhiv.rs

Paper Accepted* **ISSN Online 2406-0895**

Original Article / Оригинални рад

Vladimir Petrović [1,2,†], Biljana Radosavljević[1], Mioljub Ristić[1,2]

Seroprevalence of pertussis in adult population

Заступљеност антитела великог кашља код одраслог становништва

[1] Institute of Public Health of Vojvodina, Novi Sad, Serbia;
[2] University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia

Received: November 9, 2017
Accepted: December 5, 2017
Online First: December 8, 2017
DOI: https://doi.org/10.2298/SARH171109203P

* **Accepted papers** are articles in press that have gone through due peer review process and have been accepted for publication by the Editorial Board of the *Serbian Archives of Medicine*. They have not yet been copy edited and/or formatted in the publication house style, and the text may be changed before the final publication.
Although accepted papers do not yet have all the accompanying bibliographic details available, they can already be cited using the year of online publication and the DOI, as follows: the author's last name and initial of the first name, article title, journal title, online first publication month and year, and the DOI; e.g.: Petrović P, Jovanović J. The title of the article. Srp Arh Celok Lek. Online First, February 2017.
When the final article is assigned to volumes/issues of the journal, the Article in Press version will be removed and the final version will appear in the associated published volumes/issues of the journal. The date the article was made available online first will be carried over.

† **Correspondence to:**
Vladimir PETROVIĆ
Institute of Public Health of Vojvodina, Futoška 121, 21000 Novi Sad, Serbia
E-mail: **vladimir.petrovic@izjzv.org.rs**

Seroprevalence of pertussis in adult population

Заступљеност антитела великог кашља код одраслог становништва

SUMMARY

Introduction/Objective A seroepidemiological studies are crucial for better understanding of epidemiology of pertussis in population.

The aim of this study was to assess the seroprevalence of anti-Bordetella pertussis toxin antibodies (anti-PT IgG) in the adult population of Novi Sad, and to evaluate differences by age and gender.

Methods A cross-sectional study was conducted in 468 healthy adults aged ≥20 years stratified into seven age groups. The youngest of our participants received the last dose of the vaccine at least 18 years ago. Positive results of anti-PT IgG concentrations were considered a consequence of natural pertussis infection or reinfection. Commercial ELISA kit (Euroimmun®, Germany), with anti-PT IgG with 4 calibrators 5 IU/mL, 25 IU/mL, 100 IU/mL, and 200 IU/mL was used.

Results Most of the subjects (53.8%) had anti-PT IgG of >5 to <62.5 IU/mL. The proportion of females with high concentrations (62.5 to <125 IU/mL) were statistically significant higher than among males (5.4% vs. 0.4%, p=0.002). The highest values of anti-PT IgG were detected among subjects in the age group 20-24 years (17.5±22.2 IU/mL), and in the participants ≥60 years of age (15.0±29.4 IU/mL). The percentage anti-PT IgG concentration of ≥62.5 IU/mL was the highest among subjects aged ≥60 years (6.6%), and among those aged 20-24 years old (5.0%).

Conclusions The limited duration of vaccine induced immunity with subsequent infection or reinfection, enables the circulation of pertussis in adult population of Novi Sad that serve as the reservoirs of infection for transmission to vulnerable persons.

Keywords: pertussis; antibodies; seroprevalence; adults

САЖЕТАК

Увод/Циљ Сероепидемиолошке студије су од пресудног значаја за боље разумевање епидемиологије пертусиса у популацији.

Циљ рада био је да се процени серопреваленција антитела на *Bordetella pertussis* инфекцију (анти-ПТ ИгГ) код одраслог становништва и утврде разлике у односу на узраст и пол.

Методе Студија пресека је спроведена код 468 здравих одраслих особа, старијих од 20 година, распоређених у седам узрасних група. Најмлађи учесници студије добили су последњу дозу вакцине пре најмање 18 година. Позитивни резултати концентрација анти-ПТ ИгГ сматрани су последицама природне инфекције или реинфекције. За одређивање нивоа анти-ПТ ИгГ коришћен је комерцијални *ELISA* кит (Euroimmun®, Немачка) са 4 калибратора: 5 ИЈ/мл, 25 ИЈ/мл, 100 ИЈ/мл и 200 ИЈ/мл.

Резултати Код већине испитаника (53,8%) регистрован је ниво анти-ПТ ИгГ у распону од 5–62,5 ИЈ/мл. Статистички значајно већи проценат жена је имао високе концентрације анти-ПТ ИгГ (62,5–125 ИЈ/мл) у односу на мушкарце (5,4% наспрам 0,4%, p = 0,002). Највише вредности анти-ПТ ИгГ регистроване су код испитаника узраста 20–24 године (17,5±22,2) и узраста ≥60 година (15,0±29,4). Проценат анти-ПТ ИгГ концентрација ≥62,5 ИЈ/мл био је највиши код особа узраста ≥60 година (6,6%) и 20–24 године (5,0%).

Закључак Ограничено трајање вакциналног имунитета и следствена појава инфекција или реинфекција, омогућава одржавање пертусиса међу одраслом популацијом, који представљају резервоаре заразе за преношење инфекције на посебно осетљиве особе.

Кључне речи: велики кашаљ; антитела; серопреваленција; одрасли

INTRODUCTION

During the last decades, the incidence of pertussis has increased worldwide probably because of raised awareness, improvement in diagnostics, pathogen adaptation (a change in circulating Bordetella pertussis strains), and waning immunity after vaccination [1-3]. Available reports suggest that 16 million cases of pertussis occur annually worldwide, with 95% in low-income countries followed by 81,400 deaths [3, 4].

Substantial increase in pertussis incidence was registered among adolescents and young adults, despite high vaccination coverage [1-3]. Adults are important reservoirs for transmission of pertussis

to unvaccinated or partly immunised infants and children, who are at highest risk of severe disease and death [5, 6].

Cross-sectional serological studies are crucial for better understanding of epidemiology of pertussis in population. The IgG type pertussis toxin (PT) antibodies are specific for pertussis and do not interact with other microorganisms and are therefore measured in the serological surveys [7,8]. In countries with high immunization coverage, the high antibody levels are more likely to occur in older age groups, while in countries with lower coverage, they are more frequently detected in younger age groups [1,5,7,8]. Similar to the other parts of the world where immunization against pertussis of adults is rare, anti-PT antibodies in our serological survey could be used in adults reliably, without possibilities for detection of vaccine induced antibodies [7]. In Serbia, after primary series of vaccines (three doses during the first year of life), only one booster dose (during the second year of life) against pertussis is mandatory [9].

The main aim of this study was to assess the seroprevalence of anti-Bordetella pertussis (*B. pertussis*) toxin antibodies (anti-PT IgG) in the adult population of Novi Sad, and to evaluate differences by age and gender.

METHODS

This epidemiological, cross-sectional study was conducted by the Centre for Diseases Control and Prevention, and Centre for Microbiology of the Institute of Public Health of Vojvodina, Novi Sad. The study has been approved by the Ethics Comitee of the Institute of Public Health of Vojvodina, on 14 of May 2015 under the number 01-79/7a, as a part of wider seroepidmiological research in Vojvodina. The serum bank comprised residual sera samples of apparently healthy adults from Novi Sad, with equal representation of males and females. These individuals were stratified into seven age groups (20-24, 25-29, 30-34, 35-39, 40-49, 50-59 and ≥60 years). Serum samples from subjects were collected between 20th January 2016 and 15th June 2017. The only information available was a participant's age and gender. The size of the general population was obtained in accordance with previously published methodology [7, 10]. Our study sample included 0.17% of the population ≥20 years of age from Novi Sad as well as 0.17% of each of the seven age groups, as described in Table 1.

Anti-PT IgG concentrations were measured using a commercial ELISA kit (Euroimmun®, Germany), using anti-PT IgG with 4 calibrators 5 IU/mL, 25 IU/mL, 100 IU/mL and 200 IU/mL. The results were interpreted according to the manufacturer's recommendations.

The youngest of our participants received the last dose of the vaccine at least 18 years ago, and therefore positive results of anti-PT IgG concentrations were considered only as a consequence of natural pertussis infection or reinfection.

Statistical analysis

We classified findings into the following four groups according to the antibody levels: < 5 IU/mL, >5.0 to <62.5 IU/mL, ≥62.5 to <125 IU/mL, ≥125 IU/mL. As described in other studies [7,

11, 12, 13], anti-PT IgG levels were interpreted: ≥125 IU/mL (very high) indicates active or recent (in last 6 month) pertussis infection; ≥62.5 to <125 IU/mL (high) suggests pertussis infection during the previous 12 months; ≥5 to <62.5 IU/mL suggests exposure to pertussis infection >12 months or that the participant has never been exposed to or immunised against pertussis; <5 IU/mL considered as undetectable value indicating that the participant has never been exposed to pertussis antigens.

Gender differences in serological profile were determined using the test of proportions. Univariate analysis of variance (ANOVA) was used to explore the differences in average antibody levels according to age groups and gender. Two tailed p-values less than 0.05 were considered statistically significant.

Table 1. Blood sera samples according to age groups and gender and Anti-pertussis toxin IgG levels and pertussis seroprevalence by age group, 2017 serosurvey in Novi Sad.

Age group	Population of Novi Sad according to 2011 Census	Total number of blood sera by age (0.17% of population)	Anti-PT IgG (IU/mL) in total, mean ± SD	*p**	Number of male samples	Anti-PT IgG (IU/mL) in males, mean ± SD	*p**	Number of female samples	Anti-PT IgG (IU/mL) in females, mean ± SD	*p**
20–24	22752	40	17.5±22.2		20	17.7±18.1		20	17.2±25.6	
25–29	28646	50	10.7±16.3		25	11.4±17.4		25	10.0±15.2	
30–34	29366	50	8.4±10.4		25	8.6±8.4		25	8.2±12.1	
35-39	26299	46	6.6±7.0	0.080	23	8.5±7.5	0.336	23	4.8±5.9	0.195
40–49	46106	78	12.7±18.2		39	9.4±11.4		39	15.9±22.7	
50–59	47716	82	10.7±13.4		41	9.4±10.5		41	12.0±15.7	
60+	70967	122	15.0±29.4		61	11.0±17.7		61	19.0±37.2	
Total	**271852**	**468**	**12.1±20.2**		**234**	**10.6±14.2**		**234**	**13.6±24.7**	

* - Probability ANOVA

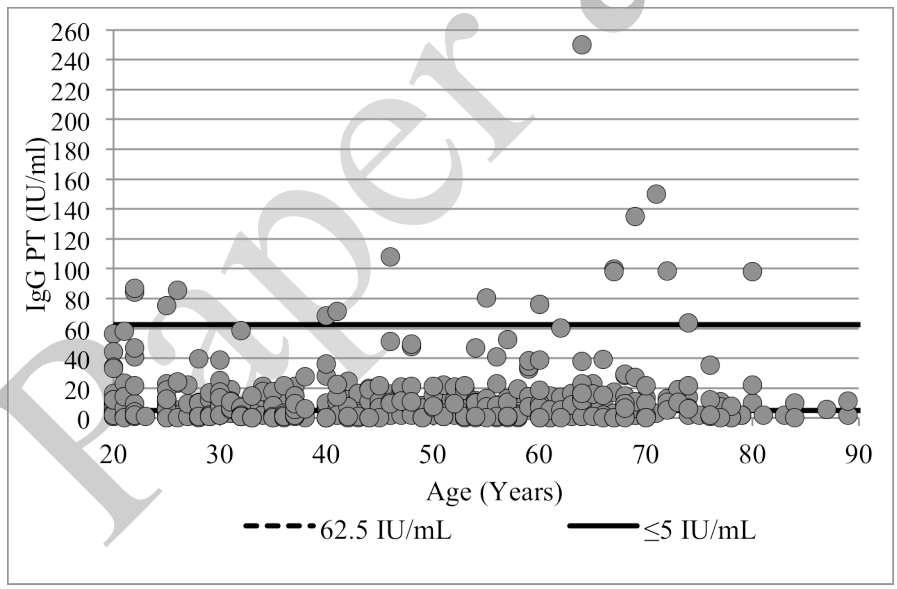

Figure 1. Distribution of anti-pertussis toxin (PT) IgG levels by age, 2017 serosurvey in Novi Sad.

RESULTS

Total of 468 blood sera from subjects ≥20 years of age from Novi Sad were analysed. The average age of the subjects was 46.4 years, the median was 45 years (IQR 32–60), and range was 20 to 89 years. The anti-PT IgG levels of the study sample ranged from 0 IU/mL to 250 IU/mL, and the median value was 10 IU/mL (IQR 3–37). Most of the subjects had anti-PT IgG level of ≥5 to <62.5 IU/mL (Figure 1).

The mean antibody level was 12.1±20.2 IU/mL (IQR 2–14). Although the mean antibody levels between the age groups were not significantly different, the highest values of anti-PT IgG were detected among subjects in the age group 20-24 years (17.5 ± 22.2 IU/mL), and in the participants ≥60 years of age (15.0 ± 29.4 IU/mL). Also, the mean anti-PT IgG levels were not significantly different between age groups, according to gender (Table 1).

There were 200 subjects (42.7%) with levels of anti-PT IgG (5 IU/mL), 252 (53.8%) had mid-range from 5.0 to <62.5 IU/mL, and 2.8% (13/468) had high levels (≥62.5 to <125 IU/mL). Three of the subjects (0.6%) had very high levels of ≥125 IU/mL. The proportion of females with high concentrations (≥62.5 to <125 IU/mL) was significantly higher than in males (5.4% vs. 0.4%, p=0.002) (Figure 2).

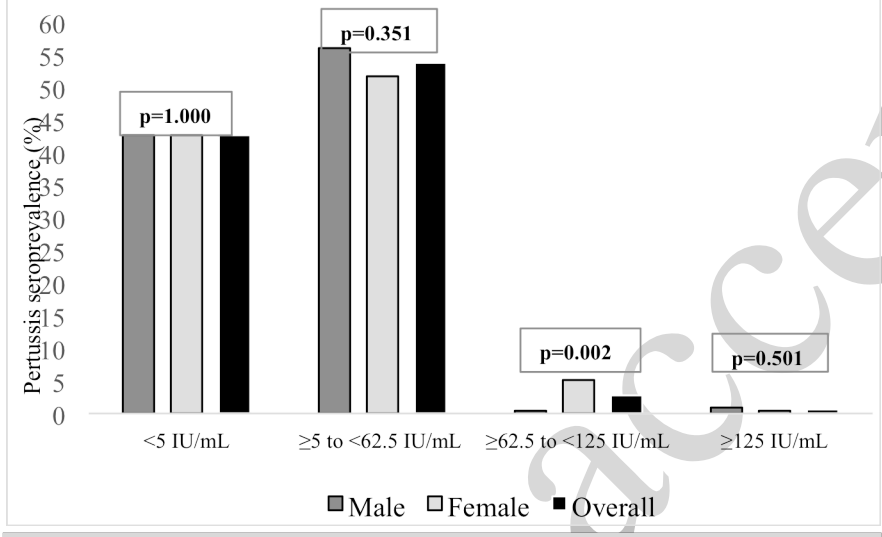

Figure 2. Distribution of anti-PT IgG levels by age and overall, 2017 serosurvey in Novi Sad.

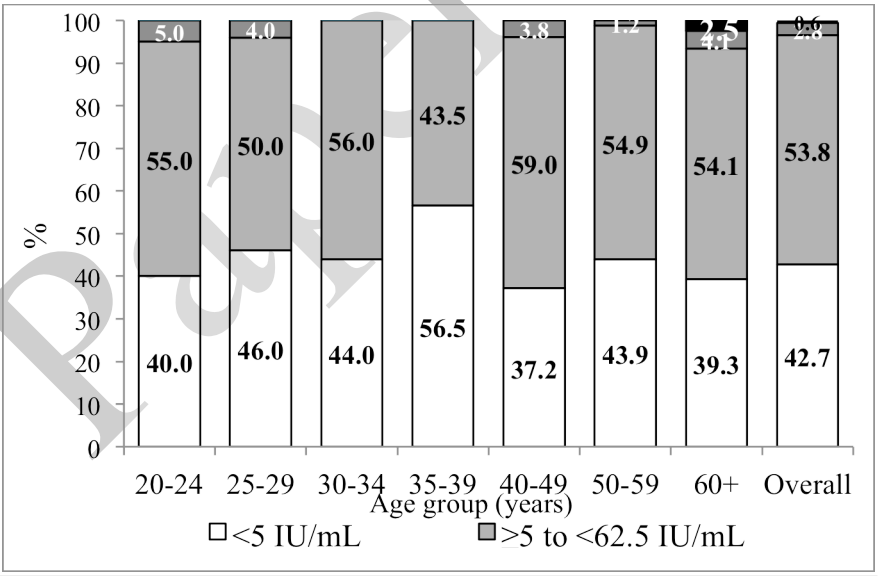

Figure 3. Concentrations of anti-pertussis toxin IgG by age group for participants aged 20 years and older (without vaccination records), 2017 serosurvey in Novi Sad.

The percentage anti-PT IgG concentration of ≥62.5 IU/mL was the highest among elderly population (6.6%), and among those aged 20-24 years old (5.0%). The high concentrations of anti-PT IgG (≥62.5 to <125 IU/mL) were detected in all age groups except in subjects aged 30-39 years old, while very high concentrations (≥125 IU/mL) were registered only among participants older than 60 years (Figure 3).

DISCUSSION

This is the first population based seropre-valence study of anti-PT IgG in our country, conducted among adults. The results of our study

highlight the presence of *B. pertussis* reservoirs in the adult population. We provided evidence that the highest values of anti-PT IgG concentrations were detected among young adults (20-24 years old), and among the elderly population (≥60 years old). In addition, we provided evidence that high anti-PT IgG concentrations were significantly higher among females than in males.

We used the commercial ELISA test (Euroimmun®) with specificity and sensitivity of 90% and 91%, respectively. The anti-PT is specific for confirmation of *B. pertussis* infection and therefore it is appropriate for seroepidemiological studies. In addition, an increased level of anti-PT IgG may be a specific marker for recent *B. pertussis* infection [7, 8, 10, 13]. Inconsistency with the results of other similar studies may in general be influenced by the use of different methods, for specificity of diagnostic cut-off values, as well as different epidemiological situation between countries.

In settings where pertussis vaccines that contain moderate to high amounts of pertussis toxin are used, vaccine-induced IgG-PT antibody concentrations decline to barely detectable levels within 2-4 years [14]. Immunity against symptomatic disease induced by acellular pertussis vaccine lasts from 4 to 7 years, and from 5 to 14 years when induced by whole cell pertussis vaccine. However, immunity after natural pertussis infection may last from 3.5 to 30 years [15, 16]. In accordance with this, the serological studies have shown a high prevalence of exposure to pertussis in adults in a variety of settings [15].

Pertussis is resurgent, and it is one of the leading cause of vaccine preventable deaths in children under five years of age [1, 2, 3, 4, 6]. The determination of age associated antibody levels against *B. pertussis* is important in deciding the target age group for booster vaccination as well as for the study of disease epidemiology [15]. Our results suggest that recent pertussis infection or reinfection after waning immunity exist among young or future parents as well as among other elderly family members, similarly as other authors have found [7, 15, 17, 18]. In our research, we find that anti-PT IgG levels ≥125 IU/mL were registered among three subjects (two men and one woman) aged over 60 years. Consistent with the results of our study, in the last two confirmed deaths among infants in Vojvodina, reported in the 1970 and 2015, the reservoirs of pertussis infection were grandparents of sick children [19, 20].

Overall, 53.8% of the adult population analysed in our study showed a detectable serological response with values of anti-PT IgG levels from ≥5.0 to <62.5 IU/mL with additional 3.4% adults who had anti-PT IgG concentration of ≥62.5 IU/mL. Considering that youngest participants received the last pertussis vaccine at least 18 years ago, we determined that most of the participants had a natural pertussis infection more than 12 months before the start of the study indicating endemicity of pertussis among adults. Nevertheless, the percentage of subjects that had a natural infection within the previous 12 months is quite high. Overall, 42.7% of the participants were susceptible for pertussis infection. Similar results have been registered among Estonian, Belgian and Danish adults [7, 21, 22].

Our findings are in agreement with previously published data that the mothers were more frequently registered as a source of transmission to infants than fathers. A Danish study found that

although the incidence in females was three times higher than in males in the 30–39 years age group, difference of anti-PT IgG levels between genders was not significant. Gender difference in incidence was explained by the fact that men seek medical care less frequently than women [22]. It is unclear whether there is a true link between pertussis and gender. However, pertussis is considered more common among females globally and whether this is due to higher immunogenicity of the female population or higher exposure to disease is yet to be defined [23, 24, 25].

Our findings recognized adults as the reservoirs of infection for pertussis for transmission to vulnerable infants, the new vaccination strategies in adults, such as the "cocooning strategy" and maternal immunization during the last trimester of pregnancy or universal immunization may be considered [18, 26-28].

CONCLUSION

The limited duration of vaccine induced immunity with subsequent infection or reinfection, enables the circulation of pertussis in adult population of Novi Sad that serve as the reservoir of infection for transmission to vulnerable subjects.

The implementation of the new immunization strategies for adults in our settings may be the proper and important approach to protect newborns, infants, and other susceptible persons from severe course of illness.

ACKNOWLEDGEMENTS

We acknowledge the staff of the Institute that provided the sera, Jelena Radovanov and Nataša Bogdanović for collecting the sera, and Milan Đilas and Predrag Pavlović for assistance in the processing and testing of sera.

The study was financially supported by the Institute of Public Health of Vojvodina.

REFERENCES

1. Edwards K, Decker MD. Whooping cough vaccine. In: Plotkin SA, Orenstein WA, Offit PA, editors. Vaccines. 6th ed. Philadelphia: Elsevier; 2013. p. 447–92.
2. Plotkin SA. The pertussis problem. Clin Infect Dis. 2014; 58(6): 830–3.
3. Pertussis vaccines: WHO position paper. Wkly Epidemiol Rec. 2010; 85: 385–400.
4. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, at al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 2012; 380(9859): 2095–128.
5. Miyashita N, Akaike H, Teranishi H, Kawai Y, Ouchi K, Kato T, et al. Diagnostic value of symptoms and laboratory data for pertussis in adolescent and adult patients. BMC Infect Dis. 2013; 13: 129.
6. Wiley KE, Zuo Y, Macartney KK, McIntyre PB. Sources of pertussis infection in young infants: a review of key evidence informing targeting of the cocoon strategy. Vaccine. 2013; 31(4): 618–25.
7. Jõgi P, Oona M, Toompere K, Lutsar I. Estimated and reported incidence of pertussis in Estonian adults: A seroepidemiological study. Vaccine. 2015; 33(38): 4756–61.
8. Guiso N, Berbers G, Fry NK, He Q, Riffelmann M, Wirsing von König CH, et al. What to do and what not to do in serological diagnosis of pertussis: recommendations from EU reference laboratories. Eur J Clin Microbiol Infect Dis. 2011; 30(3): 307–12.
9. Pravilnik o imunizaciji i načinu zaštite lekovima. Službeni glasnik Republike Srbije, 11/2006, 25/2013, 63/2013, 99/2013, 118/2013, 65/2014 i 32/2015.

10. Sočan M, Prosenc K, Vegnuti M. Seroprevalence of IgG antibodies to pertussis toxin in the Slovene population. Wien Klin Wochenschr. 2006; 118(11–12): 336–40.
11. Campbell P, McIntyre P, Quinn H, Hueston L, Gilbert GL, McVernon J. Increased population prevalence of low pertussis toxin antibody levels in young children preceding a record pertussis epidemic in Australia. PLoS One. 2012; 7(4): e35874.
12. Giammanco A, Chiarini A, Maple PA, Andrews N, Pebody R, Gay N, et al. European sero-epidemiology network: standardisation of the assay results for pertussis. Vaccine. 2003; 22(1): 112–20.
13. Jõgi P, Oona M, Toompere K, Leedo S, Epstein J, Lutsar I. Seroprevalence of IgG antibodies to pertussis toxin in children and adolescents in Estonia. Vaccine. 2014; 32(41): 5311–5.
14. de Melker HE, Versteegh FG, Schellekens JF, Teunis PF, Kretzschmar M. The incidence of Bordetella pertussis infections estimated in the population from a combination of serological surveys. J Infect. 2006; 53(2): 106–13.
15. Cagney M, MacIntyre CR, McIntyre P, Puech M, Giammanco A. The seroepidemiology of pertussis in Australia during an epidemic period. Epidemiol Infect. 2006; 134(6): 1208–16.
16. Kilgore PE, Salim AM, Zervos MJ, Schmitt HJ. Pertussis: Microbiology, Disease, Treatment, and Prevention. Clin Microbiol Rev. 2016; 29(3): 449–86.
17. Quinn HE, McIntyre PB, Backhouse JL, Gidding HF, Brotherton J, Gilbert GL. The utility of seroepidemiology for tracking trends in pertussis infection. Epidemiol Infect. 2010; 138(3): 426–33.
18. Torzsa P, Devadiga R, Tafalla M. Seroprevalence of Bordetella pertussis antibodies in adults in Hungary: results of an epidemiological cross-sectional study. BMC Infect Dis. 2017; 17(1): 242.
19. Petrović V, Durić P, Stefanović S. [Epidemiological characteristics of pertussis in Vojvodina]. Med Pregl. 2006; 59: 19–23.
20. Institute of Public Health of Vojvodina. [Communicable diseases in Vojvodina, 2015. Annual report]. Novi Sad: Institute of Public Health of Vojvodina; 2016. p. 112–16.
21. Huygen K, Rodeghiero C, Govaerts D, Leroux-Roels I, Melin P, Reynders M, at al. Bordetella pertussis seroprevalence in Belgian adults aged 20-39 years, 2012. Epidemiol Infect. 2014; 142(4): 724–8.
22. Rønn PF, Dalby T, Simonsen J, Jørgensen CS, Linneberg A, Krogfelt KA. Seroepidemiology of pertussis in a cross-sectional study of an adult general population in Denmark. Epidemiol Infect. 2014; 142(4): 729–37.
23. Skoff TH, Kenyon C, Cocoros N, Liko J, Miller L, Kudish K, at al. Sources of Infant Pertussis Infection in the United States. Pediatrics. 2015; 136(4): 635–41.
24. Jardine A, Conaty SJ, Lowbridge C, Staff M, Vally H. Who gives pertussis to infants? Source of infection for laboratory confirmed cases less than 12 months of age during an epidemic, Sydney, 2009. Commun Dis Intell Q Rep. 2010; 34(2): 116–21.
25. Sigera S, Perera J, Rasarathinam J, Samaranayake D, Ediriweera D. Seroprevalence of Bordetella pertussis specific Immunoglobulin G antibody levels among asymptomatic individuals aged 4 to 24 years: a descriptive cross sectional study from Sri Lanka. BMC Infect Dis. 2016; 16(1): 729.
26. Fedele G, Stefanelli P. Pertussis in infants and the resurgence of a vaccine preventable disease: what to do? Ann Ist Super Sanita. 2017; 53(2): 100–3.
27. Krishnaswamy S, Wallace EM, Cheng AC, Buttery J, Giles ML. Protecting newborns from pertussis: The role of partner vaccination in the era of maternal immunization. Eur J Obstet Gynecol Reprod Biol. 2017; 216: 159–63.
28. Blain AE, Lewis M, Banerjee E, Kudish K, Liko J, McGuire S, at al. An Assessment of the Cocooning Strategy for Preventing Infant Pertussis-United States, 2011. Clin Infect Dis. 2016; 63(suppl 4): S221–6.

