

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Seroprevalence of pertussis in adult population

Vladimir Petrović^{1,2}, Biljana Radosavljević¹, Mioljub Ristić^{1,2}¹Institute of Public Health of Vojvodina, Novi Sad, Serbia;²University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia**SUMMARY****Introduction/Objective** Seroepidemiological studies are crucial for better understanding of pertussis epidemiology.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 the differences by age and sex.**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. A commercial ELISA kit (Euroimmun®, Lübeck, Germany), with anti-PT IgG with four 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 participants with high concentrations (62.5 to < 125 IU/mL) was statistically significantly higher in females than in males (5.4% vs. 0.4%, $p = 0.002$). The highest values of anti-PT IgG were detected among subjects in the age group of 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 of 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 (5%).**Conclusions** The limited duration of vaccine-induced immunity with subsequent infection or reinfection enables the circulation of pertussis in the adult population of Novi Sad that serves as the reservoir of infection for transmission to vulnerable persons.**Keywords:** pertussis; antibodies; seroprevalence; adults**INTRODUCTION**

Over the last several decades, the incidence of pertussis has increased worldwide probably due to raised awareness, improvements in diagnostics, pathogen adaptation (a change in *Bordetella pertussis* strains' circulation), and waning immunity after vaccination [1, 2, 3]. Available reports suggest that 16 million cases of pertussis occur worldwide annually, with 95% in low-income countries, followed by 81,400 deaths [3, 4].

A substantial increase in pertussis incidence was registered among adolescents and young adults, despite high vaccination coverage [1, 2, 3]. Adults are important reservoirs for transmission of pertussis to unvaccinated or partly immunised infants and children, who are at the highest risk of severe form of the disease and death [5, 6].

Cross-sectional serological studies are crucial for better understanding of epidemiology of pertussis. The IgG type pertussis toxin (PT) antibodies are specific for pertussis and do not interact with other microorganisms and are therefore determined in serological surveys [7, 8]. In countries with high immunization coverage, 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 other parts of the world where immuni-

zation of adults against pertussis is rare, anti-PT antibodies in our serological survey could be used in adults reliably, without the possibility of detecting vaccine-induced antibodies [7]. In Serbia, after the 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 the differences by age and sex.

METHODS

This epidemiological, cross-sectional study was conducted by the Centre for Diseases Control and Prevention, and the Centre for Microbiology of the Institute of Public Health of Vojvodina, Novi Sad. The study has been approved by the Ethics Committee of the Institute of Public Health of Vojvodina, on May 14, 2015 under the number 01-79/7a, as a part of wider seroepidemiological 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,

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Institute of Public Health of
Vojvodina
Futoška 121
21000 Novi Sad
Serbia
vladimir.petrovic@mf.uns.ac.rs

Table 1. Blood sera samples according to age groups and sex 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 of Novi Sad)	Anti-PT IgG* (IU/mL) in total, mean \pm SD	Probability ANOVA (p)	Number of male samples	Anti-PT IgG (IU/mL) in males, mean \pm SD	Probability ANOVA (p)	Number of female samples	Anti-PT IgG (IU/mL) in females, mean \pm SD	Probability ANOVA (p)
20–24	22,752	40	17.5 \pm 22.2	0.080	20	17.7 \pm 18.1	0.336	20	17.2 \pm 25.6	0.195
25–29	28,646	50	10.7 \pm 16.3		25	11.4 \pm 17.4		25	10.0 \pm 15.2	
30–34	29,366	50	8.4 \pm 10.4		25	8.6 \pm 8.4		25	8.2 \pm 12.1	
35–39	26,299	46	6.6 \pm 7.0		23	8.5 \pm 7.5		23	4.8 \pm 5.9	
40–49	46,106	78	12.7 \pm 18.2		39	9.4 \pm 11.4		39	15.9 \pm 22.7	
50–59	47,716	82	10.7 \pm 13.4		41	9.4 \pm 10.5		41	12.0 \pm 15.7	
60+	70,967	122	15.0 \pm 29.4		61	11.0 \pm 17.7		61	19.0 \pm 37.2	
Total	271,852	468	12.1 \pm 20.2		234	10.6 \pm 14.2		234	13.6 \pm 24.7	

*Anti-PT IgG – anti-pertussis toxin IgG level; SD – standard deviation

50–59 and ≥ 60 years). Serum samples from subjects were collected between January 20, 2016 and June 15, 2017. The only information available was a participant's age and sex. The size of the general population was obtained in accordance with previously published methodology [7, 10]. Our study sample included 0.17% of the ≥ 20 years of age population 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®, Lübeck, Germany), using anti-PT IgG with four 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 to < 62.5 IU/mL, ≥ 62.5 to < 125 IU/mL, ≥ 125 IU/mL. As described in other studies, anti-PT IgG levels were interpreted as follows [7, 11, 12, 13]:

- ≥ 125 IU/mL (very high) indicates an active or recent (in the previous six 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 that exposure to pertussis infection occurred > 12 months ago or that the participant has never been exposed to or immunized against pertussis;
- < 5 IU/mL is considered an undetectable value, indicating that the participant has never been exposed to pertussis antigens.

Sex differences in serological profiles 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 sex. Two-tailed p-values < 0.05 were considered statistically significant.

RESULTS

A 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 [interquartile range (IQR) 32–60], and the range was 20–89 years. The anti-PT IgG levels of the study sample ranged 0–250 IU/mL, and the median value was 10 IU/mL (IQR 3–37). Most of the subjects had the anti-PT IgG level ranging between ≥ 5 and < 62.5 IU/mL (Figure 1).

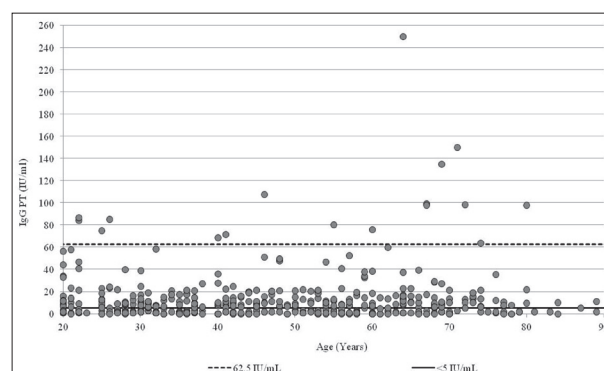


Figure 1. Distribution of anti-pertussis toxin (PT) IgG levels by age, 2017 serosurvey in Novi Sad

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 of 20–24 years (17.5 ± 22.2 IU/mL), and in the participants of ≥ 60 years of age (15.0 ± 29.4 IU/mL). In addition, the mean anti-PT IgG levels were not significantly different between the age groups, according to sex (Table 1).

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

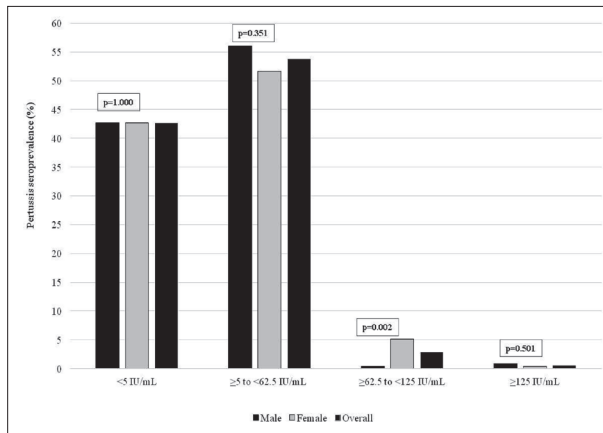


Figure 2. Distribution of anti-pertussis toxin IgG levels by age and overall, 2017 serosurvey in Novi Sad

The anti-PT IgG concentration of ≥ 62.5 IU/mL percentage was the highest among the elderly population (6.6%) and among those aged 20–24 years (5%). High concentrations of anti-PT IgG (≥ 62.5 IU/mL to < 125 IU/mL) were detected in all the age groups except in subjects aged 30–39 years, 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 seroprevalence 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 among males.

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

In settings where pertussis vaccines that contain moderate to high amounts of PT are used, vaccine-induced IgG-PT antibody concentrations decline to barely detectable levels within two to four years [14]. Immunity against symptomatic disease induced by acellular pertussis vaccine lasts from four to seven years, and from five to 14 years when induced by the whole-cell pertussis vaccine. However, immunity after natural pertussis infection may last from 3.5 to 30 years [15, 16]. In accordance with this,

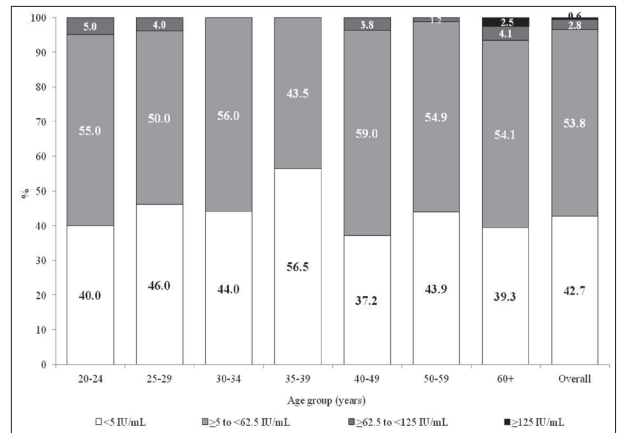


Figure 3. Concentrations of anti-pertussis toxin IgG by age group for participants aged 20 years and older (without vaccination records), 2017 serosurvey in Novi Sad

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 [1–4, 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 a recent pertussis infection or reinfection after waning immunity exists among young or future parents, as well as among other elderly family members, similar to other authors’ findings [7, 15, 17, 18]. In our research, we found 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 1970 and 2015, the reservoirs of pertussis infection were grandparents of sick children [19, 20].

Overall, 53.8% of the adult population analyzed in our study showed a detectable serological response with values of anti-PT IgG levels from ≥ 5 to < 62.5 IU/mL with additional 3.4% of adults who had anti-PT IgG concentration ≥ 62.5 IU/mL. Considering that the 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 have been registered among Estonian, Belgian, and Danish adults [7, 21, 22].

Our findings are in agreement with previously published data that 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, the difference of anti-PT IgG levels between sexes was not significant. Sex 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 sex. 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 “cooing strategy” and maternal immunization during the last trimester of pregnancy, or universal immunization may be considered [18, 26, 27, 28].

CONCLUSION

The limited duration of vaccine-induced immunity with subsequent infection or reinfection, enables the circulation

of pertussis in the 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 a severe course of the illness.

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Заступљеност антитела великог кашља код одраслог становништва

Владимир Петровић^{1,2}, Биљана Радосављевић¹, Миољуб Ристић^{1,2}

¹Институт за јавно здравље Војводине, Нови Сад, Србија;

²Универзитет у Новом Саду, Медицински факултет, Нови Сад, Србија

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 инфекцију *Bordetella pertussis* (анти-PT IgG) код одраслог становништва и утврде разлике у односу на узраст и пол.

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

Резултати Код већине испитаника (53,8%) регистрован је ниво анти-PT IgG у распону 5–62,5 IU/ml. Статистички значајно већи проценат жена имао је високе концентрације анти-PT IgG (62,5–125 IU/ml) у односу на мушкарце (5,4% наспрам 0,4%, $p = 0,002$). Највише вредности анти-PT IgG регистроване су код испитаника узраста 20–24 године ($17,5 \pm 22,2$) и узраста ≥ 60 година ($15 \pm 29,4$). Процент анти-PT IgG концентрација $\geq 62,5$ IU/ml био је највиши код особа узраста ≥ 60 година (6,6%) и у узрасту 20–24 године (5%).

Закључак Ограничено трајање вакциналног имунитета и следствена појава инфекција или реинфекција омогућавају одржавање великог кашља међу одраслом популацијом и представљају резервоаре заразе за преношење инфекције на посебно осетљиве особе.

Кључне речи: велики кашаљ; антитела; серопреваленција; одрасли