Health Prob Civil. 2025, Volume 19, Issue 4
ORIGINAL PAPER ISSN 2353-6942, eISSN 2354-0265

PART I. DISEASES AND PROBLEMS DISTINGUISHED BY WHO AND FAO

SEROLOGICAL STATUS OF MEDICAL STUDENTS OF JOHN PAUL II UNIVERSITY IN BIAŁA PODLASKA AND NURSES WORKING IN HEALTHCARE FACILITIES IN RELATION TO THE RISK OF *BORDETELLA PERTUSSIS* AND MEASLES VIRUS INFECTIONS

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Authors' contribution:
A. Study design/planning
B. Data collection/entry
C. Data analysis/statistics
D. Data interpretation
E. Preparation of manuscript
F. Literature analysis/search
G. Funds collection

Summary

Background. Considering the nature of their work and the number of hours of practical training in medical fields carried out in healthcare facilities, it is logical that nurses and medical students are particularly exposed to contact with people with infectious diseases. Therefore, the aim of this study was to determine the level of IgG anti-*B. pertussis* and anti-measles virus antibodies among medical students and practicing nurses.
Material and methods. Approximately 4 ml of venous blood was collected using closed blood collection systems from Becton Dickinson. IgG antibodies against the measles virus and *B. pertussis* toxin were measured in serum using an ELISA immunoassay (EUROIMMUN, Germany). The test kit contained microtiter plates coated with measles virus antigens or *B. pertussis*-specific toxin antigens.
Results. Positive results for antibodies against the measles virus and *B. pertussis* toxin were most common in the 18-30 age group. In other age groups, positive results were found in isolated cases.
Conclusions. As demonstrated in this study, IgG antibody levels against PT and IgG antibody levels against the measles virus decrease with age; therefore, it is necessary to continuously monitor incidence rates and control antibody levels.

Keywords: anti-PT IgG antibodies, anti-measles virus IgG antibodies, *Bordetella pertussis*, measles virus, vaccinations

Tables: 3
Figures: 0
References: 23
Submitted: 2025 Jun 24
Accepted: 2025 Aug 21
Published Online: 2025 Aug 29

Introduction

Prophylactic immunization of the largest possible number of people exerts a positive effect on population immunity and helps to reduce the prevalence of various diseases. Implementation of vaccination programs, especially those

Paszkiewicz J, Tokarska-Rodak M, Piaszczyk D, Zarębska M, Gęborys M, Ławnik A. Serological status of medical students of John Paul II University in Biała Podlaska and nurses working in healthcare facilities in relation to the risk of *Bordetella pertussis* and measles virus infections. Health Prob Civil. 2025; 19(4): 361-369. https://doi.org/10.5114/hpc.2025.153747

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against measles, pertussis, diphtheria, and tetanus, helps prevent between 2 and 3 million deaths in children annually. With increased vaccination promotion, this number may increase by another 1.5 million people [1,2]. The mandatory vaccinations against pertussis (1960) and measles (1975) introduced in Poland have contributed to a reduction in the percentage incidence of both diseases [1].

However, it should be mentioned that the first vaccinations against whooping cough used whole-cell vaccines (DTwP) containing a suspension of killed pertussis bacteria cells with an adjuvant in the form of aluminum hydroxide, containing varying amounts of all the main antigens [3]. Immunization with this type of vaccine was carried out in Poland until 2004, when an acellular vaccine (DTaP) was introduced into the Protective Vaccination Program (PSO) as a booster dose for children aged 6 years [4]. Currently, the Polish PSO includes the mandatory vaccination of children against pertussis with the DTP vaccine three times at intervals of 6 to 8 weeks in the first year of life (primary vaccination) and once in the second year of life (booster vaccination). At 6 years of age, the first booster dose is administered using the acellular DTaP vaccine, and the second booster dose, using the DTaP vaccine with a reduced dose of diphtheria toxoid and acellular components of pertussis, is administered until the age of 14 [5].

According to data from the National Institute of Hygiene (NIH) for 2023, the first dose of vaccines against pertussis and measles was received by 94.5% and 91.8% of children and adolescents, respectively. The second dose (full vaccination) was administered to only 81.2% and 88.2%. Only 82.8% of children and adolescents received a booster dose against pertussis. For comparison, in 2011, 95.8% of children and adolescents underwent a full vaccination program against pertussis, and 94.9% were fully vaccinated against measles. In turn, 96.0% and 98.1% of children and adolescents were vaccinated with booster doses against pertussis and measles, respectively. As highlighted by the recommendations of the World Health Organization (WHO), the vaccination status (two doses of a vaccine against measles) of at least 95% of the population ensures maintenance of population immunity. In the case of pertussis, the protection is sustained for approximately 10-12 years after administration of DTwP and approximately 5 years in the case of DTaP. To maintain immunity against pertussis, a booster vaccination is recommended at the age of 19 and every 10 years in adults [6].

As reported by the National Institute of Hygiene for 2010-2019, the highest prevalence of pertussis was recorded in Poland in 2016 (approximately 7,000) [4], while the highest number of cases of measles (in 2005-2021) was observed in 2019 [7,8].

The WHO data indicates that the number of reported cases of measles and pertussis has decreased significantly over the last 10 years. In 2022, Poland reported 371 cases of pertussis and 27 cases of measles. For comparison, there were 71 and 4,682 cases of measles and pertussis, respectively, in Poland in 2012. In the European region, 852 cases of measles and 5,056 cases of pertussis were reported in 2022, while the number of cases of measles and pertussis in 2012 was 27,379 and 57,539, respectively [9,10].

Therefore, monitoring public immunity to these diseases seems advisable. Due to frequent contact with sick people, medical students and healthcare workers are a special group that should be monitored for antibody levels not only against measles and pertussis. One of the methods of this control is to determine the level of IgM and IgG antibodies produced in the serum of vaccinated subjects as early as 2 weeks post-vaccination and present in the serum up to the 4th week, reaching a peak in the 3rd week. IgG antibodies reach their peak in week 3-4 after vaccination and may persist for longer [11].

Aim of the work

The purpose of the present study was to assess serum levels of anti-*B. pertussis* and anti-measles virus (MV) antibodies of the IgG class among medical students and practicing nurses.

Material and methods

The research material was blood collected from medical students at John Paul II University in Biała Podlaska and nurses working in healthcare facilities in Biała Podlaska County, Lublin Province, Poland.

The material was sampled by qualified personnel according to the recommendations for venous blood collection [12].

Approximately 4 ml of blood was drawn using Becton Dickinson closed blood collection systems. The samples were centrifuged at 1300 x g for 10 min; serum was stored at -20°C until analysis.

The study material was analyzed using serological assays. The levels of serum IgG antibodies against MV were determined with the use of an ELISA immunoenzymatic assay (Anti-Measles Virus ELISA IgG; EUROIMMUN, Germany). The kit contained reaction microwells coated with MV antigens. Inactivated Vero cell lysates infected with "Edmonston" strain MVs were the source of the antigen. The assay was performed following the manufacturer's instructions. Four calibrators as well as positive and negative controls were used for the quantitative analysis of the samples. The assay was characterized by a lower sensitivity limit of 8 IU/l and 100% sensitivity and specificity. The results were interpreted in accordance with values recommended by the manufacturer and expressed in international units (IU/l): <200 IU/l – negative result, ≥200-275 IU/l – borderline result, and ≥275 IU/l – positive result.

The titers of serum IgG antibodies against *Bordetella pertussis* toxin were determined using an ELISA immunoenzymatic assay (anti-*Bordetella pertussis* toxin ELISA IgG; EUROIMMUN, Germany). The assay was based on the *B. pertussis*-specific toxin antigen (pertussis toxin, PT) coating the reaction wells. Four calibrators as well as positive and negative controls were used for the quantitative assessment of the samples. The assay exhibited a lower limit of sensitivity of 0.2 IU/ml, 100% sensitivity, and 95.5% specificity. As specified in the manufacturer's instructions, a titer of anti-PT IgG antibodies ≥100 IU/ml was considered a positive result. Such a result may indicate either recent infection or vaccination, as vaccinated subjects may have high titers of anti-PT IgG antibodies. Repetition of the test is recommended in the case of the anti-PT IgG antibody titer in the range of ≥40-<100 IU/ml (borderline result), while an anti-PT IgG titer <40 IU/ml indicates the absence of recent infection (negative result).

The serological tests were performed, and the results were interpreted in accordance with the manufacturer's recommendations.

Photometric reading of the assay results was performed at a wavelength of 450 nm and a reference wavelength in the range of 620-650 nm using an ELISA plate reader (Thermo Scientific Multiscan FC) and the SKANIT SOFTWARE 3.1 package.

Data was statistically analyzed with the use of the Statistica v.10 program. Statistical significance was assessed using the Pearson Chi^2 test. *P*-values <0.05 were considered statistically significant.

Results

The study group consisted of 435 participants aged 18 to 77 years (mean age 41 years, SD 14.5), including 370 females (85%) and 65 males (15%). The results were analyzed in six age groups: 18-30-year-olds (138 participants, 31.7%), 31-40 year-olds (55 participants, 12.6%), 41-50-year-olds (100 participants, 23.0%),

51-60-year-olds (126 participants, 29.0%), 61-70-year-olds (13 participants, 3.0%), and over 70-year-olds (3 participants, 0.7%).

IgG antibodies against PT (anti-PT IgG) were detected with a titer qualified as a positive result in 26/435 participants (5.9%). In the vast majority of the analyzed samples (409/35 participants, 93.9%), the titer of the anti-PT IgG antibodies was <40 IU/ml (negative result).

Anti-PT IgG antibodies were detected more frequently in the group of the 18-30-year-olds, i.e. in 14/435 participants, accounting for 3.2% of all the analyzed samples (χ^2 =11.98; p=0.0350). In the other age groups, positive results were detected in only single cases, as shown in Table 1.

Table 1. IgG against PT and MV in healthy adults

Age	anti-PT IgG		MV IgG		
	Negative result N(%)	Positive result N(%)	Negative result N(%)	Borderline result N(%)	Positive result N(%)
18-30	124(28.5)	14(3.2)*	43(9.9)	23(5.3)**	72(16.6)**
31-40	51(11.7)	4 (1.0)	31(7.1)	8(1.8)	16(3.7)
41-50	99(22.7)	1(0.2)	10(2.3)	6(1.4)	84(19.3)**
51-60	121(27.8)	5(1.1)	2(0.4)	2(0.4)	122(28.0)**
61-70	11(2.5)	2(0.4)	0(0)	1(0.2)	12(2.8)
>70	3(0.7)	0(0)	0(0)	0(0)	3(0.7)
N=435(100)	409(93.9)	26 (5.9)	86(19.7)	40(9.2)	309(71.1)

Notes: PT – pertussis toxin, MV – measles virus, *p=0.0350, ** p=0.00001.

In turn, anti-MV IgG antibodies in the range of ≥275 IU/l (positive result) were detected in 309/435 participants (71.1%), and borderline results in the range of ≥200-275 IU/l were determined in 40/435 samples (9.2%).

A relationship was found between the presence of antibodies against the MV and the age of the study participants (χ^2=130.68; p=0.00001). Positive results were obtained more frequently in the age group of 18-30 years (72/435, 16.6%), 41-50 years (84/435, 19.3%), and 51-60 years (122/435, 28.0%), while borderline results were more frequent in the 18-30-year-olds (23/435, 5.3%). Detailed data is shown in Table 1.

Anti-PT IgG antibodies were detected more frequently in females aged 18-30 years, i.e. in 11/370 females (3%) (χ^2 =11.28; p=0.0460) (Table. 2).

Table 2. IgG against PT in relation to age and gender

Age	anti-PT IgG in females		anti-PT IgG in males	
	Negative result N(%)	Positive result N(%)	Negative result N(%)	Positive result N(%)
18-30	96(26.0)	11(3.0)*	28(43.1)	3(4.6)
31-40	41(11.1)	3(0.8)	10(15.4)	1(1.5)
41-50	91(24.6)	1(0.3)	8(12.3)	0(0)
51-60	111(30.0)	3(0.8)	10(15.4)	2(3.1)
61-70	9(2.4)	1(0.3)	2(3.1)	1(1.5)
>70	3(0.8)	0(0)	0(0)	0(0)
N	351(94.9)	19(5.1)	58(89.3)	7(10.7)
	370(100)		65(100)	

Notes: PT – pertussis toxin, *p=0.0460.

The borderline and positive results of anti-MV IgG antibodies in the samples collected from females were correlated with their age (χ^2 =108.78; p=0.00001). Borderline results were obtained more frequently in females aged 18-30 years, i.e. 16/370 participants (4.3%), while positive results were determined in three age groups: 18-30-year-olds, 41-50-year-olds, and 51-60-year-olds (15.7%, 20.8%, and 29.7%, respectively) (Table 3).

Table 3. Anti-MV IgG in relation to age and gender

Age	anti-MV IgG in females			anti-MV IgG in males		
	Negative result N(%)	Borderline result N(%)	Positive result N(%)	Negative result N(%)	Borderline result N(%)	Positive result N(%)
18-30	33(8.9)	16(4.3)*	58(15.7)*	10(15.4)	7(10.8)	14(21.5)
31-40	26(7.0)	4(1.1)	14(3.8)	5(7.7)	4(6.1)	2(3.1)
41-50	9(2.4)	6(1.6)	77(20.8)*	1(1.5)	0(0)	7(10.8)
51-60	2(0.5)	2(0.5)	110(29.7)*	0(0)	0(0)	12(18.5)
61-70	0(0)	1(0.3)	9(2.4)	0(0)	0(0)	3(4.6)
>70	0(0)	0(0)	3(0.8)	0(0)	0(0)	0(0)
N	70(18.9)	29(7.8)	271(73.2)	16(24.6)	11(16.9)	38(58.5)
	370(100)			65(100)		

Notes: MV – measles virus, *p=0.00001.

The simultaneous presence of anti-PT IgG and anti-MV IgG with titers qualified as positive was detected in 20/435 participants (4.6%), while 2/435 subjects (0.5%) had a positive anti-PT IgG result and a borderline anti-MV IgG result.

Discussion

It is well known that immunity to *B. pertussis*, either natural or acquired through vaccination, does not persist throughout one's lifetime and tends to decline over time [13]. In the case of measles, research suggests that immunity acquired during a bout of the disease persists throughout one's lifetime, while the protection offered by two doses of vaccines containing virus antigens decreases within 10-15 years [14].

Data reported by WHO and NIH for the years 2019-2021 indicates a downward trend in the prevalence of measles and pertussis, which requires constant control and supervision [7,8]. This is further supported by e.g. the reports of the epidemiological outbreak of measles in Italy in 2017-2018, where the number of cases amounted to 7,000 patients, with 3,263 hospitalization cases and 8 deaths [15].

According to data from the European Centers for Disease Control and Prevention (ECDC), 127 cases of measles were reported in the EU/EEA in 2022, with 59% of the total number confirmed by laboratory analyses. Measles cases were reported from 15 countries, but 77% of all the cases were detected in 5 countries (Belgium, France, Germany, Italy, and Poland). The overall notification rate was 0.3 cases per 1,000,000 inhabitants in 2022, which was lower than the rate in 2018 (34.4) and 2019 (25.4) but slightly higher than in 2021 (0.1). The notification rates in the EU/EEA countries ranged from 0 to 1.6 cases per 1,000,000 inhabitants. Their highest values were recorded in Belgium (1.6), followed by Poland (0.7), Romania (0.5), and Sweden (0.5) [16].

The results of the present study showed that 71.7% (309/435) of the examined volunteers were positive for the presence of anti-MV IgG antibodies, and 9.2% (90/435) had borderline titers.

Measles seropositivity is a humoral immune response developing in subjects who have had a measles infection or have been vaccinated against this disease. A particularly important aspect in measles prevention is the protection of newborns and infants. Therefore, it would be desirable for future mothers to receive adequate protection against measles infection, considering the statistically significant positive correlation between the levels of measles antibodies in mothers and newborns reported in literature. The higher the level of measles antibodies in the mother's serum, the longer the protection provided to newborns and infants by maternal immunity [17].

Similarly, the present study showed a relationship between the presence of antibodies and age. Positive results were obtained more frequently in the groups aged 18-30 (16.6%, 72/435), 41-50 (19.3%, 84/435), and 51-60 years (28.0%, 122/435), while borderline results were found in the 18–30-year-olds (5.3%, 23/435).

According to the National Institute of Hygiene, the initial vaccination schedule in force in Poland (1975) introduced a single dose of a single vaccine against measles (called Attenuvax) to be administered at 13-15 months of age. In 1991, two doses of a single vaccine against this disease (Rouvax) were included in the vaccination program with recommendations to be administered at 13-15 months of age and to 8-year-olds [18]. The MMR vaccine (measles, mumps, and rubella) was included in the vaccination schedule in Poland in 2004. As reported in a study conducted by Kennedy et al. in Olmsted County, MN, USA, this vaccine proved to have the highest efficacy. Kennedy et al. examined 1,025 subjects that had received the MMR vaccine. They conducted their study 7 and 17 years after the vaccination and found no decrease in humoral immunity [19].

Anichini et al., who assessed the immunization status and seroprevalence of antibodies against measles in a population of healthy adults who were vaccinated against measles or had previously been affected by the disease, obtained different results. The researchers found that 23% of the subjects that had received two doses of the vaccine exhibited no IgG response, similar to those vaccinated with only one dose (25%). In turn, a persistent high seropositivity rate was maintained in former measles patients. All participants in their study who reported a history of measles infection were seropositive [20].

Similarly, Bianchi et al. conducted a study involving 611 medical students and residents (including 201 former measles patients and 410 subjects vaccinated with two doses of MMR vaccine). They showed more durable immunity in former patients with measles than after MMR vaccination. Participants who required a booster for the MMR vaccine due to negative results accounted for 15.4% (94/611). The difference in the response to the booster dose(s) may have been associated with the longer durability of immunological memory in the former measles patients [14].

In the case of prevalence of pertussis, the latest data provided by the ECDC originates from 2018; they show that 30 EU/EEA countries reported 35,627 pertussis cases, with 93% confirmed by laboratory analyses. The notification rate was 8.2 per 100,000 inhabitants. In Poland, the notification rate was 4.1 per 100,000 inhabitants, which was the lowest rate in the country since 2014 [21].

In 2019 and 2020, Grassi et al. analyzed more than 4,154 blood serum samples collected from residents of 13 regions in Italy. The following values were adopted in their study: the lower limit of detection of anti-PTx IgG was 5 IU/ml, a borderline value of 100 IU/ml was considered to indicate a recent infection (in the year prior), and levels higher than or equal to 40 IU/ml indicated infection that had occurred over the last few years. No anti-PTx IgG antibodies (< 5 IU/ml) were detected in 38.9% of the samples. Serum samples

from 1.0% of the patients exhibited borderline values, and antibody titers between 40 and 100 IU/ml were detected in as many as 5.3% of the patients [22].

In the present study, a positive result was obtained in 5.9% (26/435) of the examined group (N=435), with the highest frequency recorded in the age group of 18-30 years (3.2%, 14/435). The investigations also highlighted an age-related downward trend in the level of anti-PT IgG antibodies. Alarming was the small number of female participants aged 18-30 years (3.0%, 11/370) and 31-40 years (0.8%, 3/370) with a positive result, i.e. in the group of women of reproductive age. A lack of maternal immunity may lead to a lack of immunity to pertussis in neonates and infants, who are especially susceptible to infections. The passive transfer of antibodies from the mother to the fetus (passive immunization) is essential for the protection of newborns and infants against pertussis in early infancy [23].

A study by Esteghamati et al. in 360 women of reproductive age showed that 33.6% of the participants did not have a protective level of antibodies, which may pose a risk of infection in neonates or infants [23].

Conclusions

Although antibody levels are not the only factor that determines immune memory, the increase in the incidence of measles and whooping cough suggests that the population's immunity to these diseases is clearly waning. As demonstrated in this study, IgG antibody levels against PT and IgG antibody levels against the MV decrease with age; therefore, it is necessary to continuously monitor incidence rates and control antibody levels.

In the study group, anti-PT IgG antibodies were detected most frequently in females aged 18-30 years (11/107), but the titers were substantially lower in the age group of 31-40 years (1/92), i.e. in women of reproductive age. Therefore, it is worth considering the introduction of mandatory serological testing or protective vaccinations in this age group.

Disclosures and acknowledgements

The authors declare no conflicts of interest with respect to the research, authorship, and/or publication of this article.

The research was funded by John Paul II University in Biała Podlaska, Poland.

Participation in the study was voluntary. Informed consent has been obtained from all the individuals included in this study. The research has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration. The consent for the study was obtained from the Bioethics Committee of John Paul II University in Biała Podlaska (Resolution 34/2017/KB/VII).

Artificial intelligence (AI) was not used in the creation of the manuscript.

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