Peer reviewed article

Seroprevalance of *Bordetella pertussis* antibodies among healthy adolescent girls in Edirne

Ülfet Vatansever[a], *Nilay Çöplü*[b], *Naci Öner*[a], *Cemile Sönmez*[b], *Serap Karasalihoğlu*[a], *Demet Kurtoğlu*[b], *Berrin Esen*[b], *Galip Ekuklu*[a]

[a] Trakya University, Faculty of Medicine, Department of Paediatrics, Edirne, Turkey
[b] Refik Saydam National HygieneCentre, Communicable Diseases Research Department, Ankara, Turkey

Summary

Objective: Immune response against pertussis can be induced by infection and/or vaccination and vaccine induced immunity is known to wane within the decade following vaccination. Our aim was to assess the pertussis immune response among adolescent girls in the province of Edirne in Turkey. In addition we determined the relationship between the immune response and age, residence, and vaccination status.

Material and methods: Serum samples were collected from 359 adolescent girls, 12 to 17 years old. The subjects were selected by systematic randomisation from school rosters and sampled by age and urban-rural residence strata proportional to the corresponding distributions in the Edirne population. Pertussis immunity was determined by an in-house quantitative ELISA method for anti-PT and anti-FHA antibodies.

Results: Protective levels of antibody (>10 EU/ml) for anti-pertussis toxin and anti-filamentous heamagglutinin were found in 95.3% and 97.2% of the overall study group respectively. In 12- to 14-year-olds protective levels were shown in 94.1% and 97.0%, in 15- to 17-year-olds in 97.5% and 97.5%, in rural areas in 96.7% and 97.5%, and in urban areas in 94.5% and 97.5%, respectively (p >0.05).

Conclusion: The high percentages of protective levels of antibodies in our study population might be an indicator of previous infections, which are a threat to infants who have not completed primary immunisation. In this respect, adult immunisation should be considered.

Key words: immunity; adolescent girls; pertussis; Turkey

Introduction

Pertussis is caused by *Bordetella pertussis* and all age groups are susceptible to this respiratory infection. However, the consequences are more severe in young children and infants [1]. It is estimated that more than 20–40 million cases and 200,000–400,000 deaths occur every year worldwide and case fatality rates in developing countries may be as high as 4% in infants [2]. To prevent this disease, vaccination is effective. Development of antibodies following vaccination with whole cell vaccine is proportionate to the number of doses administered, and the duration of immunity after vaccination is about 2–5 years. The efficacy of the vaccine varies with the content of the vaccine administered, and more than 80% of children respond to three doses of diphtheria, tetanus and whole cell pertussis (DPwT) vaccine [3]. In Turkey, whole cell pertussis vaccine is being administered in the 2nd, 3rd, and 4th months of life, in combination with a booster dose administered between the 18th and 24th months. If the booster is delayed, it can be administered to children younger than six years old. After participation in the Expanded Programme on Immunisation of the The World Health Organization (WHO), immunisation was accelerated with Turkey's "National Vaccination Campaign" in 1985. As a result of this campaign DPwT vaccination increased from 20–30% to 83%. This increased coverage of the primary pertussis vaccination decreased the incidence of disease in Turkey dramatically, from 21 cases per 100,000 in 1970 to 0.27 per 100,000 in 2001 [4, 5].

In spite of the incidence decline, the circulation of *B. pertussis* has not been eliminated, and a change in the clinical spectrum and age-related in-

The authors would like to acknowledge Trakya University Scientific Research Foundation for financial supports.

cidence of the disease has been observed [6]. There has been consistent data from a large number of countries showing an increase in the incidence of pertussis in school children, adolescents, and adults. These groups act as a source of infection for young infants who have not yet completed their primary immunisation [7, 8]. These facts led to discussions about adolescent/adult vaccination with acellular pertussis vaccines by International Consensus Group on Pertussis Immunisation, and also many country's public health policy makers [9]. Developing effective strategies for reducing the burden of pertussis must be made on a country-by-country basis, balancing need with an assessment of local circumstances.

In this study, our aim was to determine the protective antibody prevalence against two major antigens of *B. pertussis*, ie pertussis toxin (PT) and filamentous heamagglutinin (FHA), among a representative group of adolescent girls in the province of Edirne in order to provide local epidemiological data. This study, together with other national studies, was initiated to supply information vital in the discussion about adult vaccination in Turkey.

Material and methods

Study population and design

The serum samples were collected from healthy Turkish adolescent girls, aged 12 to 17, living in rural and urban areas in the province of Edirne in 2001. In 2000, the total population of Edirne was 380,000. 3.6% were adolescent girls aged 12 to 17, of whom the majority (91.4%) attended different primary and high schools [10]. In order to determine the precision of the estimation of the prevalence of pertussis seropositivity in adolescent girls, the sample size was calculated by considering reported prevalence values using the Epi Info Program [11]. In some previous studies, the prevalence of pertussis seropositivity among adolescents varied from 35% to 80% [7, 12–14]. In our study, the prevalence of pertussis seropositivity, difference and confidence interval were found to be 70%, ±5 and 95%, respectively. According to our calculation, a sample size of 320 subjects was required for the measurement of pertussis seroprevalence. However, 370 subjects were chosen to ensure accurate seropositivity measurements.

The sample size for the study was determined by a multistage sampling method that included stratification according to age (12 to 17 years) and living area and by systematic randomisation of the school rosters. The "rural areas" represented the villages and countryside, whereas the "urban areas" represented towns and the city centre. According to the population census for the year 2000, 34% of adolescent girls lived in rural areas, whereas 66% lived in urban areas [10]. All primary and high schools, in both rural and urban areas, were included in the study, and the number of students that were selected from each school was determined according to the total number of students of each school by the following formula; [(number of students per school / total number of students in Edirne) × sample size]. The next step was selecting the adolescent girls from the schools, according to their age by the following formula; [(he number of students in a particular age group / total number of adolescent girls) × sample size selected from a particular school]. Finally, the classrooms were chosen on a systematic random basis, and each adolescent girl was chosen from the selected classrooms by using a random number table. In each classroom, substitutes were selected (by a random number table), representing 50% of the number of selected adolescents. They joined the study to replace any non-participating girl from the main list of participants. The subjects were classified into two age groups: 12–14-year-olds in primary school and 15–17-year-olds in high school.

Blood samples were obtained from participants and the serum samples were stored at –20 °C until assayed.

Screening procedure and questionnaire

Following approval of the study protocol by the faculty ethics committee, the head teachers of the schools, and the local public health and education authority, adolescent girls and parents were informed about the study. Informed consent and questionnaires were obtained from both adolescent girls and their parents. The questionnaires included name, date of birth, and some demographic data such as medical history (any chronic illness or pertussis diagnosis by physician) and vaccination status. Vaccination histories were categorized into three groups; vaccinated, unvaccinated, and unknown.

Laboratory tests

ELISA tests were performed in the Refik Saydam National Hygiene Centre in 2004. 359 of the 370 serum samples were tested. 11 sera were excluded due to incorrect or insufficient serum sampling or to loss during transportation. An in-house ELISA for anti-PT and anti-FHA IgG antibodies was conducted using 96-well flat-bottom plates (Greiner, 655001, Frickenhausen, Germany). Purified PT 10 µg PN/ampoule (JNIH-5, Biken, Japan) and Purified FHA 10 µg PN/ampoule (JNIH-4, Biken, Japan) were used for coating the plates (100 ml at 0.1 µg PN/ml for PT and 0.04 µg PN/ml for FHA in 0.05 M carbonate-bicarbonate buffer, pH 9.6), which were kept in a refrigerator in humid atmosphere for 48 hours. On the test day the plates were blocked by adding 125 µl of blocking buffer (PBS containing 0.5% BSA) and incubated for one hour at 37 ºC on an Incubator/shaker (Labsystem iEM8, Helsinki, Finland). This shaker was used for every incubation period. After every step, plates were washed three times with PBS containing 0.05% Tween 20 (PBS-T). Eight two-fold serial dilutions of test sera and reference serum (anti-Pertussis Reference human sera IgG [250 ELISA Unit (EU) for anti-PT IgG, 400 EU for anti-FHA IgG, Biken, Japan]) in PBS containing 0.5% BSA and 0.05% Tween 80 were added and the following steps, which were Fc-specific alkaline phosphatase-conjugated goat anti-human IgG (Seikagaku, Kogyou, Tokyo, Japan) diluted in PBS-T; P-Nitrophenyl phosphate (Wako, Tokyo, Japan) diluted in diethanolamine buffer (1 mg/ml, pH 9.6) and 3M NaOH for stopping, were again incubated at 22 °C for one hour. Plates were read at $A_{405/630}$ on an ELISA reader (Labsystem, Multi Skan EX, Helsinki, Finland) and the anti-PT and anti-FHA IgG antibody titers were calculated by the parallel line assay ($p = 0.05$) [15, 16]. A serum with known antibody titer was included in each test run for quality control. The limit of detection for both antibodies was 1.0 EU/ml.

Evaluation

Although general agreement on the protective levels of these antibody titers measured by ELISA has not been established according to WHO, the minimum antibody level expected from vaccination is 4 EU/ml. In some other reports, 10 EU/ml was tentatively considered as the threshold, based on the lowest measured antibody titer among children recovering from pertussis [13, 17–19]. In our study, the antibody titers ≥10 EU/ml were accepted to be protective levels. Additionally, ≥100 EU/ml for anti-PT antibody was considered as recent infection [19, 20].

On the other hand, it has been shown that passively acquired anti-PT and anti-FHA antibodies have a half-life of 36 and 40 days, respectively. The geometric mean titers in cord sera were shown to be more than 150% of maternal delivery values for both of the antibodies considered [17, 21]. From this it is estimated that titers of 30 EU/ml are sufficient in mothers to protect their newborns until the first dose of vaccine is administered at 2-month-old babies in Turkey.

Statistical analysis

Statistical analysis was done using a Chi-square test in order to compare the existence of a preventive level of antibody by age group, residence and vaccination status. All calculations were performed using the Epi Info 6.04 (CDC, USA) statistical package $p < 0.05$ was considered as statistically significant.

Results

This study was conducted among 370 adolescent girls, attending 79 different secondary and high schools in Edirne, Turkey. We took blood samples from 95 selected substitutes because the subjects from the main list did not want to participate in the study, or suffered from a chronic illness; renal failure (2), malignancy (2), Type 1 Diabetes Mellitus (2), severe asthma (3) and Bruton agammaglobolinaemia (1). Eleven of the 370 blood samples were discarded and could not be analysed due to incorrect serum sampling (ie haemolysis) , insufficient serum sampling or loss during transportation. The final sample of this study included 359 adolescent girls, representing 97% of the original sample.

According to the in-house ELISA results, the antibody titers ranged between 2.35 EU/ml–1000.00 EU/ml for anti-PT and 1.40 EU/ml–1000.00 EU/ml for anti-FHA. Existence of <10 EU/ml and <30 EU/ml antibody titers for both of the antibodies varied according to the age of the subjects; the totals were 17 (4.7%) and 112 (31.2%) subjects, respectively for anti-PT antibody and 10 (2.8%) and 39 (10.9%) subjects for anti-FHA an-

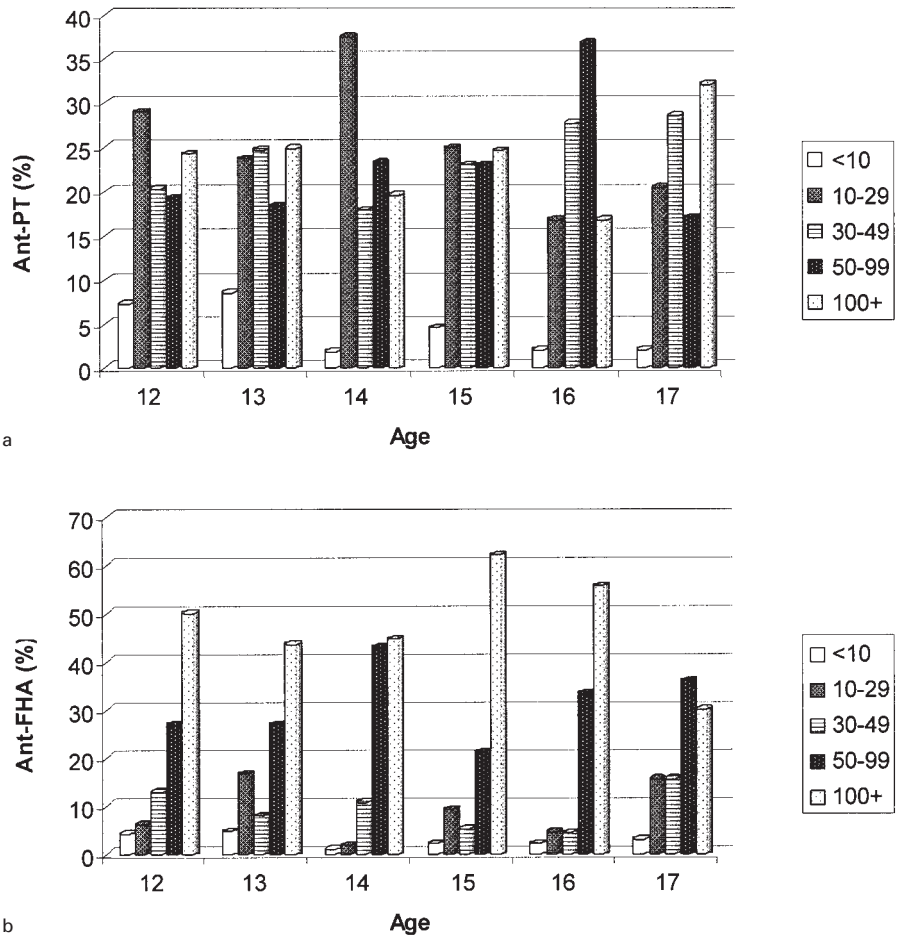


Figure 1

The distribution of pertussis anti-PT and anti-FHA antibody titers by age among adolescent girls in the province of Edirne in 2001. The antibody titers were grouped as <10 EU/ml, 10–29 EU/ml, 30–49 EU/ml, 50–99 EU/ml and ≥100 EU/ml.
a: Distribution of anti-PT antibody titer (%).
b: Distribution of anti-FHA antibody titer (%).

Table 1

Anti-PT and anti-FHA antibody levels according to age, residence and vaccination status (Edirne, 2001).

Parameters	Antibody Level (ELISA Unit/ml)											
	Anti-PT					Anti-FHA					Total	
	Non-protective Level (<10)		Protective Level (≥10)		p	Non-protective Level (<10)		Protective Level (≤10)		p		
	N	%*	N	%*		N	%*	N	%*		N	%**
Age group												
12–14	14	5.9	223	94.1		7	3.0	230	97.0		237	66.0
15–17	3	2.5	119	97.5	0.23	3	2.5	119	97.5	1.00†	122	34.0
Residence												
Rural	4	3.3	119	96.7		4	3.3	119	97.5		123	34.3
Urban	13	5.5	223	94.5	0.48	6	2.5	230	97.5	0.74	236	65.7
Vaccination status‡												
Vaccinated	15	5.2	272	94.8		8	2.8	279	97.2		287	80.0
Unvaccinated	–	–	3	100.0	1.00†	–	–	3	100.0	1.00†	3	0.8
Unknown	2	2.9	67	97.1		2	2.9	67	97.1		69	19.2
Total	17	4.7	342	95.3		10	2.8	349	97.2		359	100.0

*: Row percentage
**: Column percentage
†: Fisher's Chi-square
‡: Statistical analyses have been performed between vaccinated and unvaccinated groups.

tibody, respectively (figure 1). The existence of ≥100 EU/ml antibody titer for anti-PT antibody was 81 (22.5%).

The evaluation of the results of anti-PT and anti-FHA antibodies according to some demographic parameters is shown in table 1. The anti-PT and anti-FHA antibody titers reached protective levels in 95.3% (n = 342) and 97.2% (n = 349) of the serum samples, respectively (p >0.05). It was observed that anti-PT antibody in protective levels were lower in primary school students (94.1%) than in high school students (97.5%), but the difference was not statistically significant (p >0.05). Similarly, urban and rural residents had no statistically significant difference, although the percentages were 94.5% and 96.7%, respectively (p >0.05). Protective levels of anti-FHA antibodies were found in more than 97% in both age and residence groups (p >0.05) (table 1).

When the vaccination status of the subjects was evaluated, three subjects had not been vaccinated in the past. However, they all showed protective levels of antibodies (100%). These subjects had no history of either having a pertussis infection or a chronic disease diagnosed by a physician. Protective levels of anti-PT and anti-FHA antibodies among the vaccinated girls were 94.8% and 97.2%, respectively (p >0.05).

Discussion

Although pertussis infection has been controlled by mass vaccination in infancy and early childhood, there has been a shift in the age distribution of infection. Moreover, clinical symptoms have changed because of the limited duration of protective levels of antibody following vaccination. Passively acquired maternal antibodies protect infants, but waning levels of antibody may increase the vulnerability of infants. Infection of adolescents and adults can then become a threat for infants who have not completed their primary pertussis immunisation series [17, 21]. In order to take appropriate preventive health care measures against pertussis, including vaccination by acellular vaccine, there is a need to determine the incidence of pertussis in each country.

Our study examined the antibody levels in adolescent girls in the province of Edirne by using an in-house ELISA to determine the prevalence of anti-PT and anti-FHA antibodies, quantitatively. ELISA tests are widely used for detection of antibodies against PT and FHA [13, 16, 19–21]. The procedure of the in-house ELISA test performed in this study was compared to a Ball ELISA (Takeda Chemical Industries, LTD., Osaka, Japan) which has previously been studied and found to be reliable. The correlation coefficients between the two methods were 0.776 and 0.729 for anti-FHA and anti-PT, respectively, and regression coefficients were 0.639 and 0.623, respectively (unpublished data) [22–23]. Consequently, the results have been applied to a seroepidemiological study in the province of Samsun, Turkey, and the distributions of the antibodies have been found to be reasonable [12]. In this study protective levels of anti-PT and anti-FHA antibodies were found in

more than 95% and 97% of the study population, respectively [12]. Additionally, the three unvaccinated subjects in our study also had protective levels of antibodies (table 1). The latter is difficult to explain by immunisation when the vaccination route in Turkey is taken into consideration. Furthermore, the percentage of anti-PT ≥100 EU/ml was 22.5% and indicated recent infection [19, 20]. The findings suggest natural contact with *B. pertussis*. Although infection is mild in adults and there is a small number of susceptible subjects in this study group, the circulation of the organism caries a risk for newborns who should be protected against a life-threatening pertussis infection. According to our results, nearly ⅓ of expected mothers will have babies with low titers of passive immunity and subsequently, may become infected (figure 1). On the other hand, antibody titers of young mothers that were vaccinated during their adolescence may be too low to protect their infants prior to completion of the routine vaccination series. In order to overcome such problems, some authors suggest vaccination during pregnancy, and others suggest immunisation of household contacts, including siblings [21, 24, 25]. In addition, some experts suggest that maintenance of pertussis immunity requires additional boosters for adolescents and adults to lower the incidence of disease and to prevent further spreading of the disease [9, 24, 26, 27]. Conversely, the use of additional doses needs to be evaluated to determine the cost-benefit ratio and the impact on the epidemiology of pertussis, especially on the age distribution of the disease. The additional resources required and the likelihood of continued availability of these resources should be carefully analysed prior to implementing such schedules [17].

Results of similar epidemiological studies from other countries have demonstrated a discrepancy of protective levels of antibodies between 35% and 90%, which is in accordance with our results [7, 13, 14, 26]. In the province of Samsun, Turkey, anti-PT and anti-FHA antibodies higher than ≥10 EU/ml in adolescents were 77.4% and 77.4% respectively [12]. The difference in percentages may be due to a lower frequency of the circulation of the organism.

Other similar studies found no statistically significant difference between males and females. This suggests that our data could be interpreted for the entire adolescent population in Edirne [14, 26].

In conclusion, this study demonstrated the occurrence of natural pertussis exposure among adolescent girls and highlighted the threat of infection among infants. Additional vaccination of adolescents and adults for the prevention of infant infections needs to be considered.

Acknowledgments: The authors would like to acknowledge the contributions of Dr. Hülya Tuğrul Beyzadeoğlu, Dr. Sebahattin Şit, Dr. Güner Emel Yolsal, Dr. Hülya Karaca, Dr. Evrim Seçkin, Dr. Sema Can, and Dr. Güler Ateş in regional data collection and handling.

Correspondence:
Ülfet Vatansever, MD
Trakya University
Faculty of Medicine, Department of Pediatrics
TR-22030 Edirne
Türkiye
E-Mail: uvatansever@trakya.edu.tr

References

1 Mortimer EA. Pertussis. In Krugman's Infectious diseases children. Katz SL, Gershon AA, Hotez PJ (eds). 10th ed. Mosby-Year Book, Inc, Missouri.1998, 335–49.
2 Pertussis Vaccines. WER 1999;74:137–44. http://www.who.int/vaccines/en/pertussis.html.
3 Petersen JW. Cellular immunity in relation to pertussis vaccination and infection. Danish Medical Bulletin 1995;42:121–40.
4 General Directorate of Primary Health Care of Ministry of Health, Turkey. The Annual of Statistics 2001. Table 40. www.saglik.gov.tr/extras/istatistikler/temel2001/103.html.
5 General Directorate of Primary Health Care of Ministry of Health, Turkey. The Annual of Statistics 2001. Table 38, www.saglik.gov.tr/extras/istatistikler/temel2001/100.html.
6 Taranger J, Trollfors B, Bergfors E, et al. Mass vaccination of children with pertussis toxoid decreased incidence in both vaccinated and nonvaccinated persons. Clin Infect Dis 2001;33:1004–9.
7 Arav-Boger R, Ashkenazi S, Gdalevich M, Cohen D, Danon YL. Seroprevalence of pertussis antibodies among adolescents in Israel. Isr Med Assoc J 2000;2:174–7.
8 Cattaneo L, Reed G, Haase D, Wills M, Edwards K. The seroepidemiology of Bordetella pertussis infections: a study of persons ages 1–65 years. J Infect Dis 1996;173:1256–9.
9 Campins-Marti M, Cheng HK, Forsyth K, et al. Recommendations are needed for adolescent and adult pertussis immunisation: Rationale and strategies for considerations. Vaccine 2002;20:641–6.
10 2000 Turkey Census of Population. State Institute of Statistics Prime Ministry Republic of Turkey, Printing Division, Ankara, 2002.
11 Epi info (computer program). Revision 1. Atlanta: Centers for Disease Control and Prevention; 2002.
12 Ministry of Health of Turkey: National epidemiological surveillance of vaccine preventable diseases based on laboratory data. Turkish Bull Hyg Exp Biol 2001;58(Suppl. 1).
13 Corbeira PG, Dal Ra R, Aguilar L, Garcia-de-Lomas J. Seroepidemiology of Bordetella pertussis infections in the Spanish population: a cross-sectional study. Vaccine 2000;18:2173–6.
14 Dominguez A, Vidal J, Plans P, Salleras L. The seroepidemiology of B.pertussis infection in Catalonia, Spain. Epidemiol Infect 2001;126:205–10.
15 Finney DJ. Statistical Method in Biological Assay. Charles Griffin Co. Ltd., London, 1987.
16 Relyveld E, Oata NH, Nuet M, Gupta RK. Determination of antibodies to pertussis toxin in working reference preperations of anti-pertussis sera from various national control laboratories. Biologicals 1992:20:67–71.
17 Immunological basis for immunization. Module 4: Pertussis WHO/EPI/GEN/98.14.
18 Konda T, Kamachi K, Iwaki M, Matsunaga Y. Distribution of pertussis antibodies among different age groups in Japan. Vaccine 2002;20:1711–7.

19 Giammanco A, Chiarini A, Maple PAC, et al. European Ser-Epidemiology Network: Standardisation of the assay results for pertussis. Vaccine 2003,22:112–20.
20 de Malker HE, Versteegh FGA, Conyn-van Spaendonck MAE, et al. Specificity and sensitivity of high levels of immunoglobulin G antibodies against pertussis toxin in a single serum sample for diagnosis of infection with Bordetella pertussis. J Clin Microbiol 2000;38:800–6.
21 Healy CM, Munoz FM, Rench MA, Halasa NB, Edwards KM, Baker CJ. Prevalence of pertussis antibodies in maternal delivery, cord, and infant serum. J Infect Dis 2004;190:335–40.
22 Sato Y, Sato H, Kodama H, et al. An improved ELISA system for the measurement of IgG antibodies against pertussis toxin (PT) and filamentous hemagglutinin (FHA) in human sera. Develop Biol Standart 1990;73:167–74.
23 Kuno-Sakai H, Kimura M, Ohta K, et al. A simple and sensitive ELISA of antibodies to pertussis antigens. Vaccine 1992;10: 350–3.
24 Van Rie A, Hethcote HW. Adolescent and adult pertussis vaccination: computer simulations of five new strategies. Vaccine 2004;22:3154–65.
25 Edwards KM. Pertussis: an importanat target for maternal immunization. Vaccine 2003;21:3483–6.
26 Wielen MV, Damme PV, Herck KV, Schlegel-Haueter S, Siegrist CA. Seroprevalence of Bordetella pertussis antibodies in Flanders (Belgium). Vaccine 2003;21:2412–7.
27 Buydner PGV, Owen D, Vurdien JE, Andrews NJ, Matthews RC, Miller E. Bordetella pertuss›s surveillance in England and Wales: 1995–7. Epidemiol Infect 1999;123:403–11.

Swiss
Medical Weekly

Official journal of
the Swiss Society of Infectious disease
the Swiss Society of Internal Medicine
the Swiss Respiratory Society

The many reasons why you should choose SMW to publish your research

What Swiss Medical Weekly has to offer:

- SMW's impact factor has been steadily rising, to the current 1.537
- Open access to the publication via the Internet, therefore wide audience and impact
- Rapid listing in Medline
- LinkOut-button from PubMed with link to the full text website http://www.smw.ch (direct link from each SMW record in PubMed)

- No-nonsense submission – you submit a single copy of your manuscript by e-mail attachment
- Peer review based on a broad spectrum of international academic referees
- Assistance of our professional statistician for every article with statistical analyses

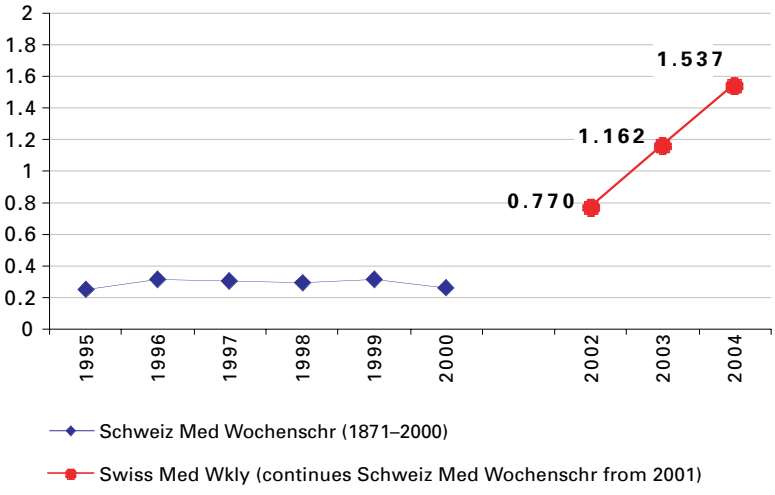
- Fast peer review, by e-mail exchange with the referees
- Prompt decisions based on weekly conferences of the Editorial Board
- Prompt notification on the status of your manuscript by e-mail
- Professional English copy editing
- No page charges and attractive colour offprints at no extra cost

We evaluate manuscripts of broad clinical interest from all specialities, including experimental medicine and clinical investigation.

We look forward to receiving your paper!

Guidelines for authors:
http://www.smw.ch/set_authors.html

Impact factor Swiss Medical Weekly



EMH FMH Schwabe
Editores Medicorum Helveticorum

All manuscripts should be sent in electronic form, to:

EMH Swiss Medical Publishers Ltd.
SMW Editorial Secretariat
Farnsburgerstrasse 8
CH-4132 Muttenz

Manuscripts: submission@smw.ch
Letters to the editor: letters@smw.ch
Editorial Board: red@smw.ch
Internet: http://www.smw.ch