Arch. Immunol. Ther. Exp. (2015) 63:155–160
DOI 10.1007/s00005-014-0314-x

SHORT COMMUNICATION

Colonization of *Bordetella pertussis* Clinical Isolates that Differ by Pulsed Field Gel Electrophoresis Types in the Lungs of Naïve Mice or Mice Immunized with the Whole-Cell Pertussis Vaccine Used in Poland

Maciej Polak · Monika Zawadka · Ewa Mosiej · Daniel Rabczenko · Ewa Augustynowicz · Nicole Guiso · Anna Lutyńska

Received: 13 March 2014 / Accepted: 18 June 2014 / Published online: 9 October 2014

Abstract The goal of our study was to compare the elimination of *Bordetella pertussis* clinical isolates that differ according to pulsed field gel electrophoresis (PFGE), serotypes and genes encoding virulence factors from the lungs of naïve mice or mice immunized with commercial diphtheria-tetanus-whole-cell pertussis vaccine used in Poland. When a mixture of four isolates, given in equal proportions and harboring different PFGE profiles, serotypes, and alleles encoding virulence factors, was used to infect non-immunized mice, a single isolate, characterized by PFGE type IVγ, Fim2 phenotype and *ptxA1–prn2–tcfA2–fim2-1–ptxP1–ptxC1–fim3-1* alleles, was found to be significantly predominant compared to the others. This PFGE profile is commonly found in *B. pertussis* isolates circulating in some European countries since the late 1990s, confirming its high fitness. The Polish commercial whole-cell pertussis vaccine induced an immunity effective at eliminating the *B. pertussis* isolates from the lungs. However, the elimination of the isolate harboring PFGE type C profile, Fim2,3 phenotype and *ptxA1–prn1–tcfA2–fim2-1–ptxP1–ptxC1–fim3-1* alleles was delayed as compared to the others, suggesting phenotypic differences with the other isolates and vaccine strains. Nevertheless, the same isolate, when challenged into mice in the defined mixture of strains, lost the competition with the others, as measured by lung colonization efficiency. This PFGE profile represents 15 % of the isolates circulating in Poland between 2001 and 2012.

Keywords *Bordetella pertussis* · Whole-cell pertussis vaccine · PFGE · Pertussis toxin S1 subunit · Pertactin · Mouse model of infection

Introduction

Whooping cough, caused by *Bordetella pertussis* and *Bordetella parapertussis*, is a highly transmissible, vaccine-preventable disease that results in significant rates of morbidity in unvaccinated individuals (Mattoo and Cherry 2005). The rates of whooping cough have been significantly reduced in countries that maintain high immunization coverage with whole-cell pertussis (wP) vaccines used generally since the 1950s (Mattoo and Cherry 2005). Acellular pertussis (aP) vaccines that contain purified *B. pertussis* antigen(s) have been gradually introduced into European and other developed countries vaccination schedules in place of wP vaccines because of their more acceptable safety profile. These aP vaccines contain detoxified pertussis toxin (Ptx) alone or are combined with one adhesin [filamentous hemagglutinin (FHA)], two adhesins [FHA and pertactin (Prn), or four adhesins [FHA, Prn and fimbrial proteins (Fim)]. In these

Electronic supplementary material The online version of this article (doi:10.1007/s00005-014-0314-x) contains supplementary material, which is available to authorized users.

M. Polak · M. Zawadka · E. Mosiej · E. Augustynowicz · A. Lutyńska (✉)
Department of Sera and Vaccines Evaluation, National Institute of Public Health - National Institute of Hygiene, Warsaw, Poland
e-mail: alutynska@pzh.gov.pl

D. Rabczenko
Department - Centre for Monitoring and Analyses of Population Health Status and Health Care System, National Institute of Public Health - National Institute of Hygiene, Warsaw, Poland

N. Guiso
Molecular Prevention and Therapy of Human Diseases Unit, Institut Pasteur, Paris, France

highly vaccinated countries reported resurgence of pertussis has been regarded as an important public health problem since the 1990s (Celentano et al. 2005; Tan et al. 2005; Zepp et al. 2011). The waning of immunity and pathogen adaptation was basically regarded to be responsible for re-emergence of pertussis (Hegerle and Guiso 2013; Mooi et al. 1998, 2014). The routine use of aP vaccines was also hypothesized as the force for the appearance of Prn-deficient *B. pertussis* isolates (Bouchez et al. 2009; Hegerle et al. 2012).

Poland has seen a resurgence of pertussis since the late 1990s, although children have been continuously immunized since 1960 with three primary doses and one booster dose at 2, 3–4, 5–6 and 16–18 months of age with a nationally produced diphtheria–tetanus–whole-cell pertussis (DTwP) vaccine (Gzyl et al. 2004b). An booster dose of DTaP for 6-year-old children was introduced in 2004 to prevent the waning of immunity, which has been observed in schoolchildren since the late 1990s (Gzyl et al. 2004b; Zieliński et al. 2004).

Genetic changes were observed in the *B. pertussis* isolates circulating in Europe since the 1990s. It was shown that isolates currently circulating in developed countries express $Fim3^{+}$ and are part of PFGE group IV (including subgroups IVα or BpSR11, IVβ or BpSR10 and IVγ or BpSR12) and harbor *ptxA1–prn2* alleles in their genome but this is not the case in low vaccine coverage countries such as Senegal (Hegerle et al. 2012). The pathogen adaptation due to vaccine pressure hypothesis pioneered by Frits Mooi's team (Mooi et al. 1998) was further supported by their discovery of a novel allele for the pertussis toxin promoter (*ptxP3*), associated with increased pertussis toxin production (Mooi et al. 2009). Currently *ptxP3* allele harboring isolates became dominating in some European countries and were shown to be absent from Africa where immunization was introduced later than Europe and where vaccine coverage is relatively low.

Currently, Poland is a single EU country still using the same locally produced wP vaccine in children up to 2 years old (Gzyl et al. 2004c; Zawadka et al. 2014). Genetic changes observed in the *B. pertussis* isolates circulating in Poland seem to be driven slightly differently compared to elsewhere in Europe (Mosiej et al. 2011). Between 1997 and 2000, around 55 % of Polish *B. pertussis* isolates were found to be part of new PFGE groups A and C (Mosiej et al. 2011). However, those new PFGE groups are comprised of isolates that harbor similar alleles of the S1 subunit of Ptx (*ptxA1*), the promoter of the Ptx locus (*ptxP1*) and Prn (*prn1* or *prn2*).

In the present study, our objective was to analyze, using the murine model of infection, whether (1) the isolates belonging to different PFGE groups in relation to serotypes and alleles encoding virulence factors, colonize the mice differently, and (2) immunity induced by the commercial Polish wP vaccine is able to eliminate these isolates from the lungs of the vaccinated mice. We chose clinical isolates collected between 1997 and 2000 because during this period an increase of pertussis was observed in Europe, generally. This choice was in line with suggestions that antigenic divergence between vaccine strains and clinical isolates with respect to *prn* and *ptxA* may have contributed to the resurgence of pertussis (Mooi et al. 1998). Within a period of 1997–2000, isolates belonging to new PFGE groups and producing different Prn (type 1, 2 or 3) were collected (Hegerle and Guiso 2013; Mooi et al. 2014). It was then hypothesized that pertussis resurgence was due to the circulation of these isolates (Hegerle and Guiso 2013; Mooi et al. 2014). Furthermore, in Poland, we identified new PFGE groups that have not been found in other countries yet.

Materials and Methods

Bacterial Strains

In this study, the clinical *B. pertussis* isolates 1758/97, 1797/98, 939/98, 2316/99, 1203/00, and one of the vaccine seed strain of commercially available Polish DTwP vaccine-606/67 were used (Table 1). All *B. pertussis* in the collection were stored at −70 °C using seed bank system, minimizing the possibility of passage adaptation. The bacteria were cultured on Bordetella Selective Medium (BSM, Oxoid, UK) and incubated at 37 °C for 2–3 days. Their PFGE profiles, serotypes and *ptxA, prn* alleles were previously described (Mosiej et al. 2011). In addition, genes encoding Ptx S3 subunit (*ptxC*), tracheal colonization factor (*tcfA*), Fim2 and 3 (*fim2*, *fim3*) and Ptx promoter were identified by sequencing with the primers and conditions described (Mooi et al. 2009; van Loo et al. 2002).

Vaccine

The locally produced, Polish commercial DTwP vaccine (DTP, IBSS Biomed S.A., Poland) was used. *B. pertussis* vaccine seed strains used for the DTwP vaccine productions were changed several times (Gzyl et al. 2004a). The last change of vaccine seed strains composition took place in 1978 and was aimed at eliminating strains expressing the highest toxicity. Since then, the wP component of the Polish DTwP vaccine has been produced with three vaccine seed strains, 606/67, 629/65 and 186/65. All the vaccine seed strains belong to the PFGE group III and harbor the same *ptxA2–ptxC1–ptxP1–prn1–tcfA2–fim2-1–*

Table 1 Characterization of *B. pertussis* isolates under study

Strain				PFGE	Allele							Serotype
No.	Collection	Isolation year	Remarks									
606/67	NIPH-NIH	1967	Vaccine strain	III	*ptxA2*	*prn1*	*tcfA2*	*fim2-1*	*ptxP1*	*ptxC1*	*fim3-1*	Fim2,3
1203/00	NIPH-NIH	2000	Clinical isolate	III	*ptxA1*	*prn1*	*tcfA2*	*fim2-2*	*ptxP1*	*ptxC1*	*fim3-1*	Fim2
2316/99	NIPH-NIH	1999	Clinical isolate	IVγ	*ptxA1*	*prn2*	*tcfA2*	*fim2-1*	*ptxP1*	*ptxC1*	*fim3-1*	Fim2
1758/97	NMI	1997	Clinical isolate	V	*ptxA1*	*prn3*	*tcfA3*	*fim2-1*	*ptxP1*	*ptxC1*	*fim3-1*	Fim2,3
1797/98	NMI	1998	Clinical isolate	A	*ptxA1*	*prn1*	*tcfA2*	*fim2-1*	*ptxP1*	*ptxC1*	*fim3-1*	Fim2
939/98	NIPH-NIH	1998	Clinical isolate	C	*ptxA1*	*prn1*	*tcfA2*	*fim2-1*	*ptxP1*	*ptxC1*	*fim3-1*	Fim2,3

NIPH-NIH National Institute of Public Health - National Institute of Hygiene, Warsaw, Poland, *NMI* National Medicines Institute, Warsaw, Poland

fim3-1 profile and Fim2,3 serotype (Table 1) (Mosiej et al. 2011; Zawadka et al. 2014). Because of these similarities, we have chosen only one of the vaccine seed strain (606/67) for the study.

Intranasal Challenge Model

The animal protocol was approved by the Ethical Committee for Experiments in Animals. Four-week-old female BALB/c mice (MMRC, Warsaw) were immunized twice with i.p. injections of 0.25 single human dose of the Polish commercial wP vaccine at 2-week intervals. Two weeks after the second injection, the mice were intranasally challenged with 50 µl of a bacterial suspension containing approximately 5×10^7 colony forming units (CFU) *B. pertussis*. Mice immunized with commercially available DTwP vaccine used in Poland were challenged with *B. pertussis* differentiated by PFGE types: III (606/67 or 1203/00), IVγ (2316/99), V (1758/97), A (1797/98), C (939/98), or the mixture of isolates belonging to PFGE groups III, IVγ, V, C (606/67, 2316/99, 1758/97, 939/98). The isolates were chosen according their prevalence in their PFGE groups between 1997 and 2000. The PFGE profiles of the isolates were representative for Europe (PFGE groups IV and V as high and low prevalence), Poland (PFGE group A and C) and vaccine seed strains of DTwP used in Poland (PFGE group III). Details on serotypes and allele genes studied are presented in the Table 1. The outcome of the challenge was followed by performing CFU counts on lung homogenates (homogenized in 1 ml casein hydrolysate per lung at 3,000 rpm for 3 min) cultured on BSM plates from individual mice (three per time point), sacrificed after 2 h (day 0) and 5, 8 and 12 days after the challenge. The results are reported as $\log_{10}$ CFU obtained in the mice lungs per time point. The $\log_{10}$ values during the whole course of infection were used to compare DTwP vaccine efficiency in the elimination of *B. pertussis*. The limit of detection of $\log_{10}$ 0.85 was found to be equivalent with CFU values of zero in all samples.

Statistical Analysis

The log-transformed CFU in each mouse and on each day were compared using two-way ANOVA with random effects. In the case of obtaining significant results, the Tukey multiple comparison procedure was applied to identify pairs of significantly influencing challenges. Analysis was done using R 2.15.0 statistical program; random-effect ANOVA was computed using lme function from the nlme package (Pinheiro et al. 2013). Tukey multiple comparison procedure was computed using glht function from the multcomp package (Hothorn et al. 2008).

Analysis of the Mixed Infections

The proportions of four strains (606/67, 2316/99, 1758/97, 939/98) in the challenge mixture given to immunized and naïve mice were determined on days 1, 3/4 and day 15/18/19 post challenge. These time points were chosen according to the presence of bacterial growth in the lungs. At each time point, up to 30 single colonies (28.5 and 29.5 average and median, respectively) were randomly picked up from plates of the lowest dilutions of lung homogenates and grown on BSM. PFGE for the genetic characterization of *B. pertussis* isolates was performed according to the standardized procedure (Advani et al. 2004). The PFGE profiles of the single colonies were identified using GelCompar Software (Applied Maths) and compared with profiles previously determined for 606/67, 939/98, 2316/99 and 1758/97 isolates. The significance of the increase or decrease in the number of a particular isolate initially sustaining one-fourth of the challenge mixture between days was determined using extended Mantel–Haenszel test for nominal categories (Greenland 1989). Computations were done using WinPepi program (Abramson 2011).

Results

Bacterial Characterization According to the PFGE Profiles, Serotypes and Genotyping

The PFGE profiles, serotypes and alleles harbored by *B. pertussis* under study are described in Table 1. All isolates harbor the same *ptxP1*, *ptxC1* and *fim3-1* alleles. Vaccine strain 606/67 belongs to the PFGE group III, similar to clinical isolate 1203/00, but they harbor different serotypes (Fim2,3 and Fim2, respectively) and *ptxA* and *fim2* alleles (*ptxA2*, *fim2-1* and *ptxA1*, *fim2-2*, respectively). Isolates 1758/97 and 2316/99 belonging to PFGE groups V and IVγ differed by serotype (Fim2,3 and Fim2, respectively) and *prn, tcfA* allele profiles (*prn3*, *tcfA3*, and *prn2*, *tcfA2*, respectively). The isolates 1797/98 and 939/98 assigned to PFGE group A and C, not found previously in other European countries, harbored an identical allele profile of *ptxA1*, *prn1*, *ptxP1*, *ptxC1*, *tcfA2*, *fim2-1* and *fim3-1*, but different serotype (Fim2 and Fim2,3, respectively).

Repeatability of the Intranasal Challenge Model

Repeatability of the model was verified by determination of the homogeneity of challenge suspensions and homogeneity of experimental infections. In the lungs, the average *B. pertussis* $\log_{10}$ CFU ± SD values for the control and immunized mice at day 0 reached 5.99 (±0.51) and 5.73 ± 0.57, respectively. The differences in the CFU counts for the challenge doses before the infection and the differences in the CFU counts for the challenge suspensions given to the mice on day 0, 2 h after challenge, expressed as CV %, were lower than 11 %.

Two independently performed experiments in DTwP-immunized mice challenged with a single isolate (606/67) or the mixture of four isolates did not reveal statistically significant differences ($p = 0.938$ and 0.863, respectively). Repeatability was also confirmed by the lack of statistical differences among CFU counts obtained in five experiments performed in control mice challenged with the same mixture strains ($p < 0.7259$). Since the model was found to be reproducible and for ethical reasons, all of the other experiments were performed once.

Elimination of *B. pertussis* from Mice Immunized with Commercial DTwP Vaccine Used in Poland and Challenged Individually or with the Mixture of Isolates

Using the murine intranasal challenge model, we evaluated the effectiveness of the commercial locally produced DTwP vaccine to eliminate the different isolates that belong to different PFGE, serotypes and alleles profile groups, given individually or as a mixture. As expected, significant differences between immunized and control groups were observed at 5 ($p < 0.001$), 8 ($p < 0.001$) and 12 days ($p < 0.001$) post infection (Fig. 1; Supplementary Table 1). DTwP vaccine produced with strains of PFGE type III, Fim2,3 phenotype and *ptxA2–prn1–tcfA2–fim2-1–ptxP1–ptxC1–fim3-1* alleles was effective in eliminating all five isolates given individually or as a mixture (Fig. 1). However, the elimination of one isolate harboring PFGE type C, Fim2,3 phenotype and *ptxA1–prn1–tcfA2–fim2-1–ptxP1–ptxC1–fim3-1* alleles was delayed as shown in Fig. 1. This delay is significant at day 5 and 8 (*p* value from 0.0059 to <0.001).

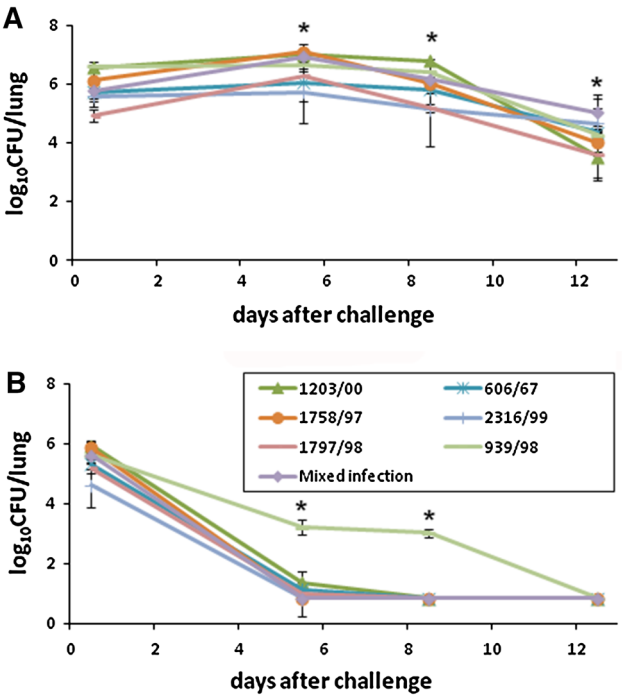

Fig. 1 Lung clearance of the *B. pertussis* isolates belonging to different PFGE groups in non-immunized (**a**) and immunized with DTwP mice (**b**). Results are shown as mean values of CFU per lung ($\log_{10}$-transformed) ± SD. The limit of detection $\log_{10}$ 0.85 is the equivalent to all samples with CFU values of zero. *Asterisks* in **a** represent significant differences between control groups and immunized groups ($p < 0.001$), and in **b** differences between mice challenged with 939/98 and mice challenged with other isolates (*p* value from 0.0059 to <0.001)

Distribution of *B. pertussis* Recovered during the Course of Infection Induced by a Mixture of Isolates that Differ According to their PFGE Group

To study bacterial colonization, the changes in the proportion of bacteria belonging to different PFGE, serotypes and alleles profile groups were monitored after infection

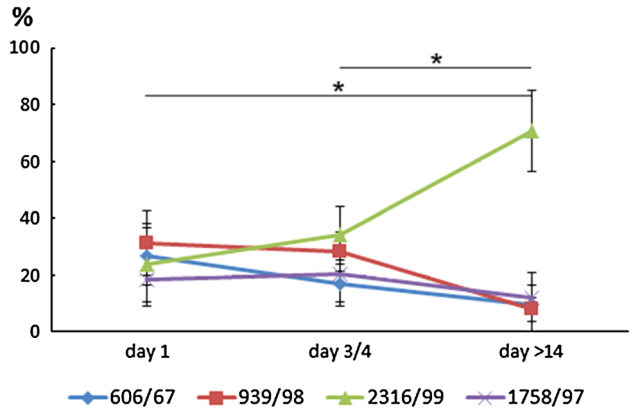

Fig. 2 The proportions of four isolates (606/67, 2316/99, 1758/97, 939/98) in the naïve mice lungs after challenge with the mixture composed of equal quantities of 606/67, 2316/99, 1758/97, and 939/98 isolates determined on days 1, 3/4 and 15/18/19. These time points were chosen according to the presence of bacterial growth in the lungs. Results are shown as means of four independent experiments. *Asterisks* indicate significant differences ($p < 0.001$)

with a mixture of these bacteria within the course of infection up to 15, 18 or 19 days post infection. Statistically significant differences were observed only in the control mice. The proportion of the four isolates, 606/67 (PFGE III–Fim2,3–*ptxA2–prn1–tcfA2–fim2-1–ptxP1–ptxC1–fim3-1*), 939/98 (PFGE C–Fim2,3–*ptxA1–prn1–tcfA2–fim2-1–ptxP1–ptxC1–fim3-1*), 2316/99 (PFGE IVγ–Fim2–*ptxA1–prn2–tcfA2–fim2-1–ptxP1–ptxC1–fim3-1*) and 1758/97 (PFGE V–Fim2,3–*ptxA1–prn3–tcfA3–fim2-1–ptxP1–ptxC1–fim3-1*), in the lungs of naïve mice is shown in Fig. 2. The proportion of the four types of bacteria in the lungs after 14 days from the challenge differs statistically when compared to days 1 or 3/4. The strain 2316/99 (PFGE IVγ–Fim2–*ptxA1–prn2–tcfA2–fim2-1–ptxP1–ptxC1–fim3-1*) became statistically predominant compared to the others after 14 days of infection ($p < 0.001$) and comprised 71 % of the bacterial population analyzed.

Discussion

B. pertussis is considered to be a pathogen undergoing a process of adaptation, mainly in the view of findings regarding mismatches in sequence changes for several genes encoding important antigens and differences on the genome level (Hegerle and Guiso 2013; Mooi et al. 2014). The adaptation of bacteria due to long-term vaccination pressure and waning immunity are suggested as possible factors responsible for the resurgence of pertussis (Mooi et al. 2014). Since specific *B. pertussis* PFGE groups have been shown to circulate in Poland (Mosiej et al. 2011), we aimed to compare them with other PFGE groups circulating in Europe between 1997 and 2000 using the in vivo mice model, to analyze whether differences between them can be observed.

The immunity induced after vaccination of the mice using the Polish commercial wP vaccine did eliminate the bacteria from the lungs of infected mice. However, the elimination of one isolate belonging to a specific Polish PFGE group (group C) and harboring Fim2,3 serotype and alleles profile *ptxA1–prn1–tcfA2–fim2-1–ptxP1–ptxC1–fim3-1* has been delayed. The prevalence of newly emerged PFGE group C, found 32 % between 1997 and 2000, might have resulted of pathogen adaptation and waning immunity potentiated by the lack of a pertussis vaccine booster up to 2004 in Poland. This stay in line with widely accepted concept of main reasons of pertussis resurgence (Hegerle and Guiso 2013; Mooi et al. 2014). The immunity induced by the Polish wP vaccine was able to eliminate quickly the bacteria belonging to the other PFGE group circulating in Poland (group A), carrying Fim2 phenotype and alleles profile *ptxA1–prn1–tcfA2–fim2-1–ptxP1–ptxC1–fim3-1* and the bacteria belonging to the other PFGE group circulating predominantly in Europe (group IV) carrying Fim2 phenotype and alleles profile *ptxA1–prn2–tcfA2–fim2-1–ptxP1–ptxC1–fim3-1*. Differences in PFGE groups but not in a *ptxA–prn* allele profiles in bacteria under study were responsible for the delay observed. This confirms the importance of the PFGE technique to compare circulating isolates.

Differences in the elimination of *B. pertussis* isolates from Poland were previously found (Gzyl et al. 2004a), however, in the present study were confirmed using more appropriate statistical analysis. We investigated at which level the bacteria belonging to PFGE group C behave differently than the others. Anyway, prevalence of PFGE group C isolates, together with introduction of child booster, decreased up to 15 % since 2004 (data unpublished), confirming that this group of isolates can be controlled if herd immunity is increased or due to strain frequency dependent selection.

In the second part of this study we analyzed the fitness of a mixture of four isolates belonging to different PFGE groups, serotypes and carrying different *ptxA–prn–tcfA* alleles. We observed that after infection one type of bacteria predominates over the others during the infection. The isolate of PFGE group IVγ, Fim2 phenotype and alleles *ptxA1–prn2–tcfA2–fim2-1–ptxP1–ptxC1–fim3-1* winning the competition with the others in vivo (PFGE group III–Fim2,3 phenotype–*ptxA2–prn1–tcfA2–fim2-1–ptxP1–ptxC1–fim3-1*, PFGE group V–Fim2,3 phenotype–*ptxA1–prn3–tcfA3–fim2-1–ptxP1–ptxC1–fim3-1* and PFGE group C–Fim2,3 phenotype–*ptxA1–prn1–tcfA2–fim2-1–ptxP1–ptxC1–fim3-1*), might be regarded as a one of confirmed increased fitness. One can hypothesize that this

isolate might be more successful when adaptive immunity is lacking. However, this isolate was similarly eliminated from the lungs of immunized mice compared with the others when it was inoculated to the mice individually.

In their last published study, van Gent et al. (2011) reported that the ability to colonize mice decreased in the order Prn1 > Prn2 and Prn3. Our results clearly show that differences in colonization, are determined not only by the *prn* allele, but by other factors somehow unconnected to *prn* or *ptxA* genes. It indicates that not only genomic studies but also proteomic studies are important to find out at which level the differences between isolates are located. Furthermore, studies performed with different *B. pertussis* strains, in the experimental conditions of competition using defined mixtures, in vivo, as used in our study, might lead to the identification of those of real advantage features.

Acknowledgments We would like to express our gratitude to Arno van der Ark (RIVM, The Netherlands) for critical analysis of the manuscript. We would like to also express our appreciation to Mrs Barbara Husejnow for her excellent technical assistance. This work was supported by the Ministry of Science and Higher Education in Poland (grant no. N N404 206839).

References

Abramson JH (2011) WINPEPI updated: computer programs for epidemiologists, and their teaching potential. Epidemiol Perspect Innov 8:1

Advani A, Donnelly D, Hallander H (2004) Reference system for characterization of Bordetella pertussis pulsed-field gel electrophoresis profiles. J Clin Microbiol 42:2890–2897

Bouchez V, Brun D, Cantinelli T et al (2009) First report and detailed characterization of *B. pertussis* isolates not expressing Pertussis Toxin or Pertactin. Vaccine 27:6034–6041

Celentano LP, Massari M, Paramatti D et al (2005) Resurgence of pertussis in Europe. Pediatr Infect Dis J 24:761–765

Greenland S (1989) Generalized Mantel-Haenszel estimators for K 2 x J tables. Biometrics 45:183–191

Gzyl A, Augustynowicz E, Gniadek G et al (2004a) Sequence variation in pertussis S1 subunit toxin and pertussis genes in *Bordetella pertussis* strains used for the whole-cell pertussis vaccine produced in Poland since 1960: efficiency of the DTwP vaccine-induced immunity against currently circulating *B. pertussis* isolates. Vaccine 22:2122–2128

Gzyl A, Augustynowicz E, Rabczenko D et al (2004b) Pertussis in Poland. Int J Epidemiol 33:358–365

Gzyl A, Augustynowicz E, Rabczenko D et al (2004c) Potency of pertussis component in the DTP vaccine—an overview of three decade study in Poland. Biologicals 32:129–137

Hegerle N, Guiso N (2013) Epidemiology of whooping cough and typing of *Bordetella pertussis*. Future Microbiol 8:1391–1403

Hegerle N, Paris A-S, Brun D et al (2012) Evolution of French *Bordetella pertussis* and *Bordetella parapertussis* isolates: increase of Bordetellae not expressing pertactin. Clin Microbiol Infect 18:E340–E346

Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. Biom J 50:346–363

Mattoo S, Cherry JD (2005) Molecular pathogenesis, epidemiology, and clinical manifestations of respiratory infections due to *Bordetella pertussis* and other *Bordetella* subspecies. Clin Microbiol Rev 18:326–382

Mooi FR, van Oirschot H, Heuvelman K et al (1998) Polymorphism in the *Bordetella pertussis* virulence factors P.69/pertactin and pertussis toxin in The Netherlands: temporal trends and evidence for vaccine-driven evolution. Infect Immun 66:670–675

Mooi FR, van Loo IH, van Gent M et al (2009) *Bordetella pertussis* strains with increased toxin production associated with pertussis resurgence. Emerg Infect Dis 15:1206–1213

Mooi FR, van der Maas NA, de Melker HE (2014) Pertussis resurgence: waning immunity and pathogen adaptation—two sides of the same coin. Epidemiol Infect 142:685–694

Mosiej E, Augustynowicz E, Zawadka M et al (2011) Strain variation among *Bordetella pertussis* isolates circulating in Poland after 50 years of whole-cell pertussis vaccine use. J Clin Microbiol 49:1452–1457

Pinheiro J, Bates D, DebRoy S et al (2013) R Development Core Team. nlme: linear and nonlinear mixed effects models. R package version 3.1-113. R Development Core Team, Vienna

Tan T, Trindade E, Skowronski D (2005) Epidemiology of pertussis. Pediatr Infect Dis J 24(5 suppl):S10–S18

Van Gent M, van Loo IHM, Heuvelman KJ et al (2011) Studies on Prn variation in the mouse model and comparison with epidemiological data. PLoS One 6:e18014

Van Loo IHM, Heuvelman KJ, King AJ et al (2002) Multilocus sequence typing of *Bordetella pertussis* based on surface protein genes. J Clin Microbiol 40:1994–2001

Zawadka M, Mosiej E, Polak M et al (2014) Consistency of *Bordetella pertussis* vaccine seed strains and potency of whole-cell pertussis vaccine still in use in Poland. Biologicals 42:123–127

Zepp F, Heininger U, Mertsola J et al (2011) Rationale for pertussis booster vaccination throughout life in Europe. Lancet Infect Dis 11:557–570

Zieliński A, Rosińska M, Czarkowski M et al (2004) The effectiveness of vaccination with whole-cell pertussis vaccine by age group in Poland 1996-2001. Scand J Infect Dis 36:114–118