Effect of the inclusion of „contemporary" *B. pertussis* strains in the vaccine composition on temporal trends in *B. pertussis* population

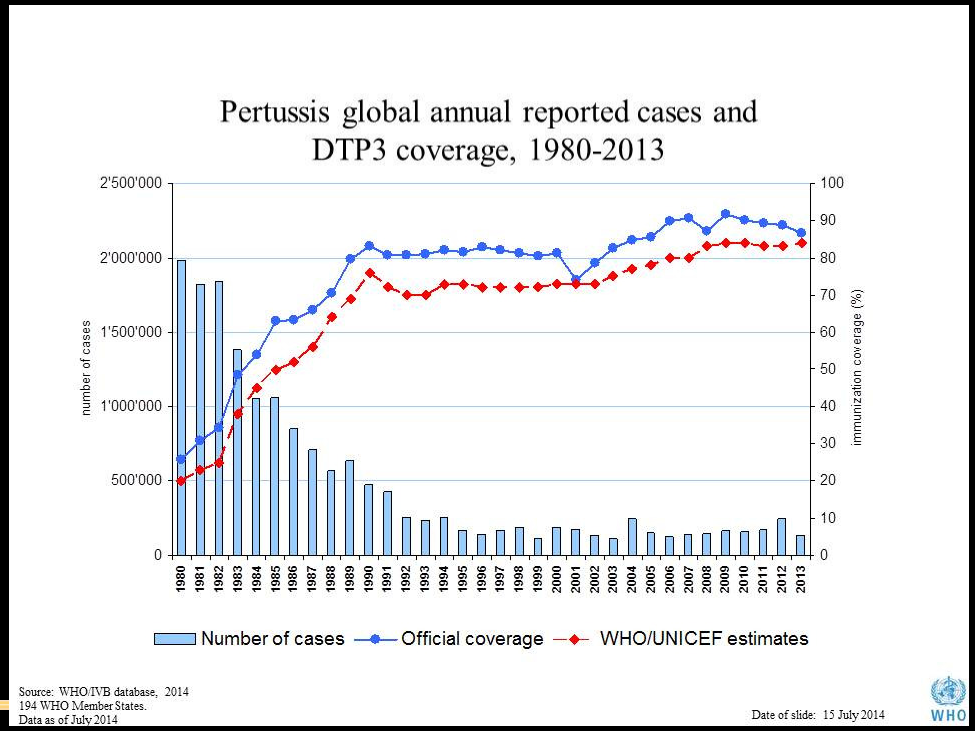
Tatjana Plješa, MD, PhD
Institute of Virology, Vaccines and Sera –Torlak
Belgrade, Serbia

- **Pertussis or whooping cough has persisted and resurged in the face of vaccination and has become one of the most prevalent vaccine-preventable diseases in Western countries with estimated infection frequencies of 1–9% (Mooi et al, 2013)**
- ***Bordetella pertussis* poses a threat to infants that have not been (completely) vaccinated and for whom pertussis is a severe, life-threatening, disease (de Greeff, 2010)**



Pertusis - one of the leading causes of vaccine preventable deaths in the world today

WHO, 2013.	
Estimated cases	16. 000. 000
Estimated deaths	89. 000
Vaccine coverage	84%



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Resurgence in countries with long vaccination history and high coverage

- Argentina (Hozbor et al., 2009)
- Canada (Skowronski et al., 2002; Ntezayabo et al., 2003)
- USA (Yih et al., 2000; CDC, 2002a; CDC, 2003a; Tanaka et al., 2003)
- Australia (McIntyre et al., 2002; Spokes and Gilmour, 2011)
- Netherland (de Melker et al., 2000)
- Israel (Moerman et al., 2006)
- Spain (Crespo et al., 2011)
- Finland (Elomaa et al., 2005)
- UK (Litt et al., 2009) etc.

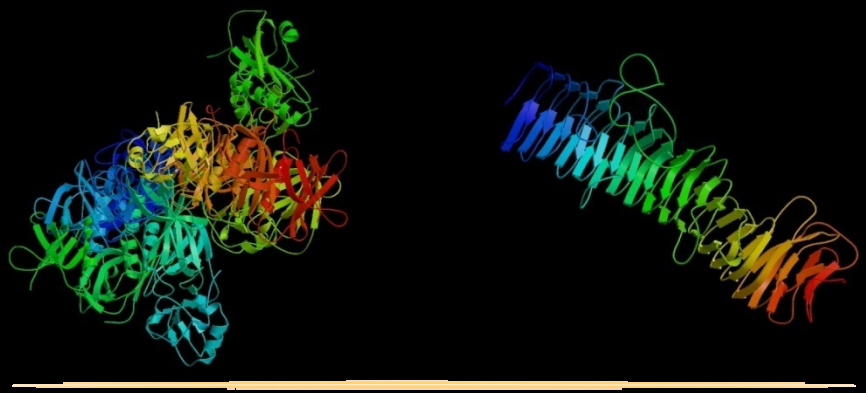
?

Resurgence of pertussis –possible reasons

- Better education and awareness about disease (He and Mertsola, 2008)
- Better diagnostic methods & improved reporting (He and Mertsola, 2008)
- Waning of adaptive immunity through time (Wendelboe et al, 2005)
- Adaptation to the vaccine induced immunity (He and Mertsola, 2008)
- WCVs induce longer lasting immunity than ACVs, the switch from WCVs to ACVs may have aggravated the pertussis problem (Gustafsson, 2006; Sheridan, 2012)
- Antigenic divergence of Ptx and Prn (He and Mertsola, 2008; Mooi et al, 2013)

Polimorphism of nucleotides

- *B. pertussis* virulence factor show polymorphisms
- Wide spread antigenic divergence between circulating isolates and vaccine strains
- Alleles changes from vaccine to non-vaccine types – every 15-30 years



Pertussis toxin

Pertactin

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Polimorphism of pertussis toxin

- 5 types of PtxA:

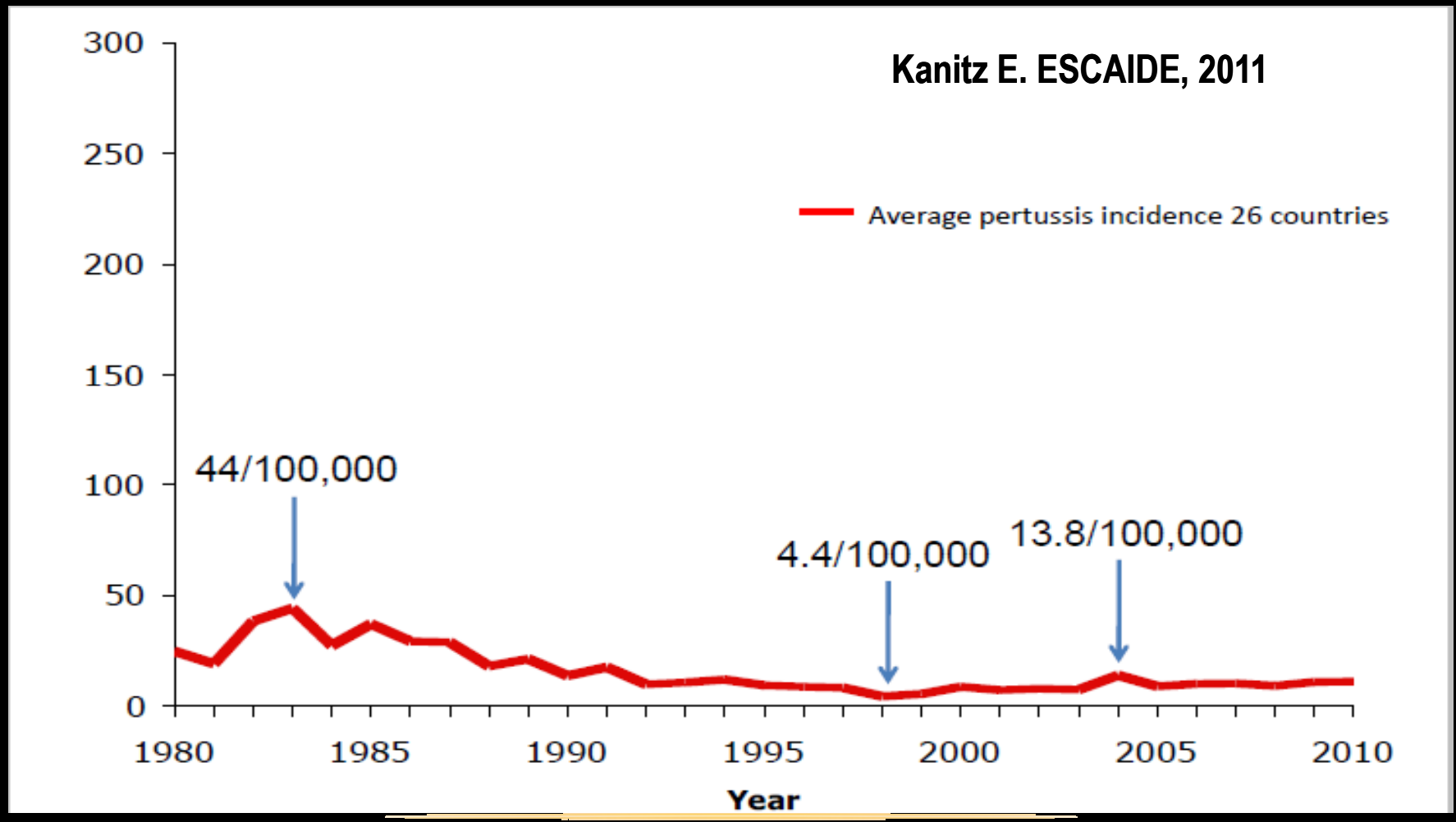
  PtxA1, PtxA2, PtxA4, PtxA5 and PtxA8 (Mooi et al., 2010).

- Predominant in isolates – PtxA1 and PtxA2 (Mooi et al., 2013)
- Most vaccines – PtxA1, PtxA2 and PtxA4 (Litt et al., 2009)
- Tohama (widely use acelular vaccine strain) - PtxA2

Polimorphism of pertactin

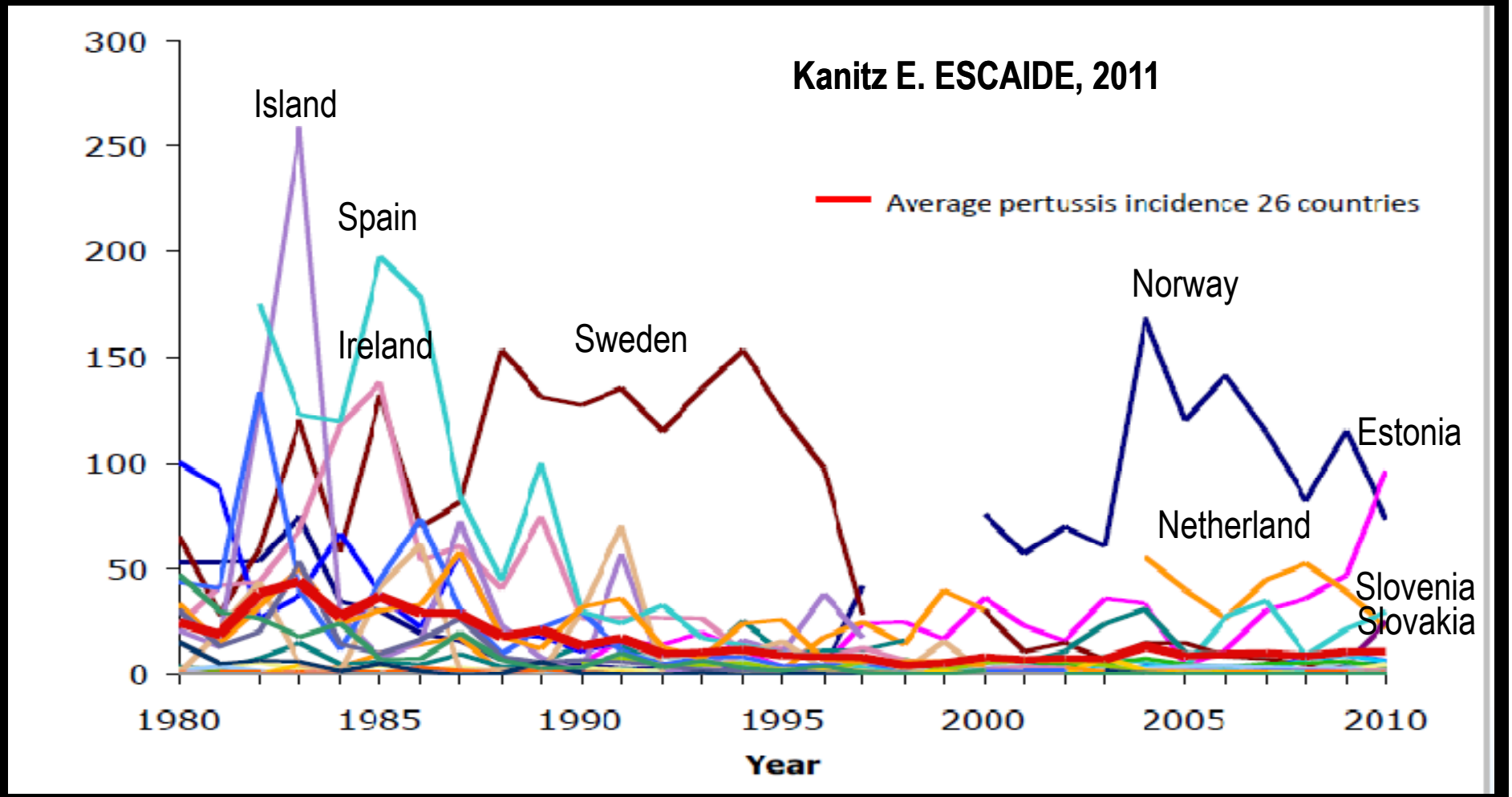
- 13 different pertactin allels
- Alleles changes from vaccine to non-vaccine types – every 15-30 years
- Predominant in isolates: Prn1, Prn2 and Prn3 (Mooi et al., 2010)
- Vaccine strains : Prn1, Prn7 and Prn10 (Mooi et al., 2010)

Average pertussis incidence in 26 European countries



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Pertussis incidence in European countries



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Vaccine types

wP-containing:

- Inactivated whole bacterial cell
- Different strains
- Isolates as vaccine strains
- Both celular and humoral immune response
- Th1 type & Th17

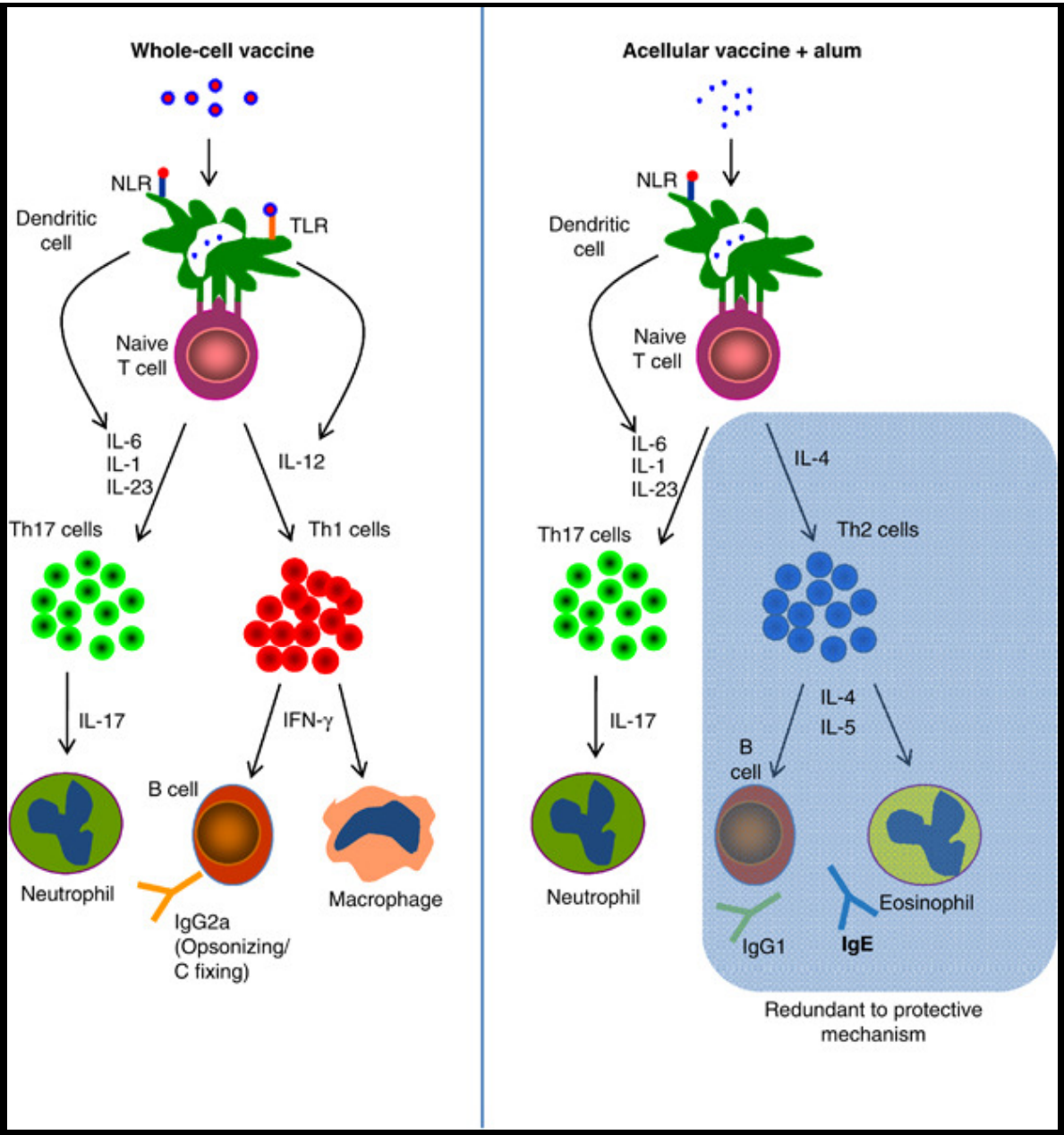
aP-containing:

- PT ; PT & FHA; PT, FHA & PRN; PT, FHA, PRN, Fim2 & Fim3
- Tohama vaccine strain
- Humoral imune response
- Th2 type & Th17

Vaccine safety

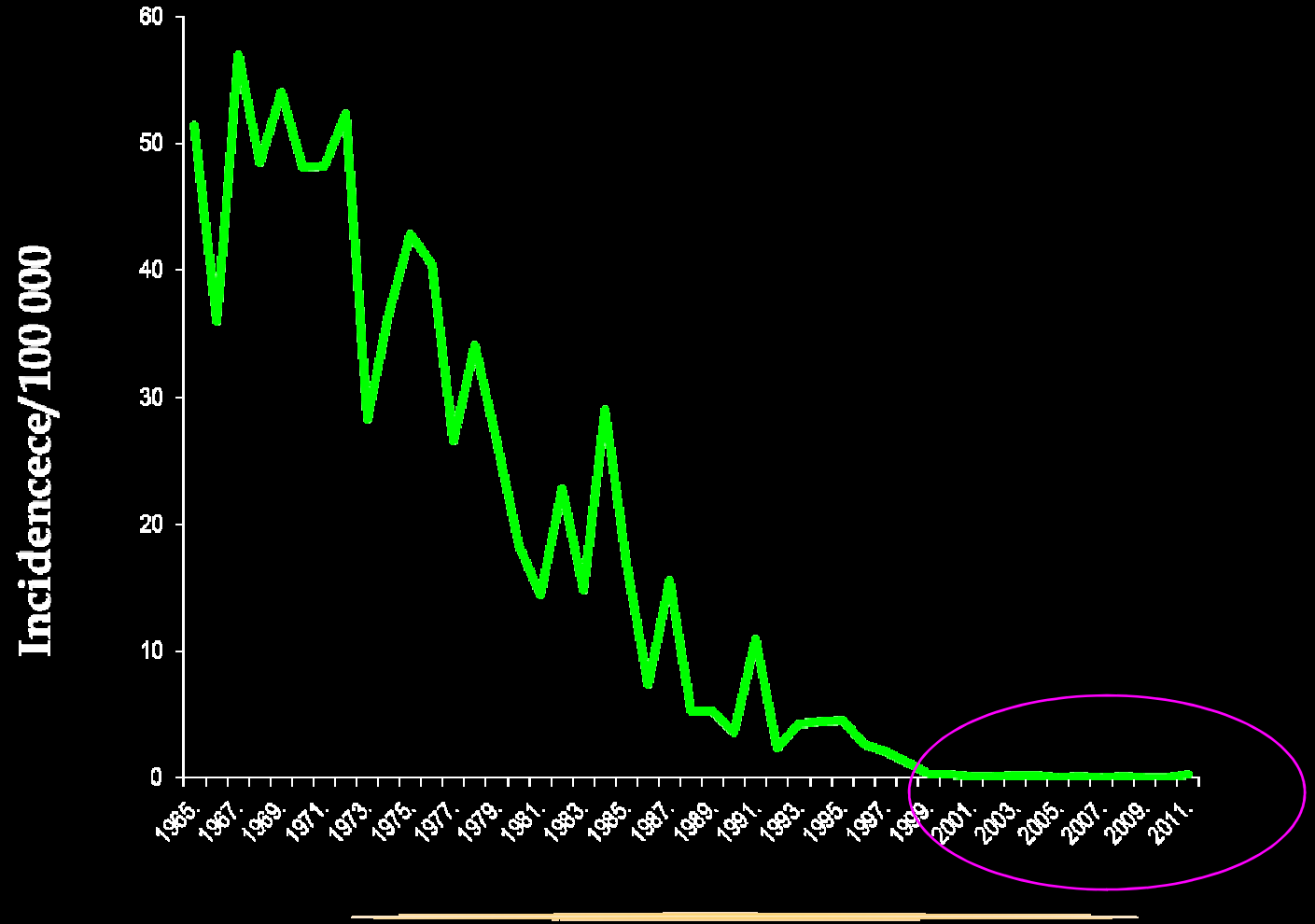
Although local and systemic reactogenicity are more commonly associated with wP-containing vaccines, both aP-containing and wP-containing vaccines have excellent safety records.

WHO, Weekly epidemiological record, No. 30, 25 july 2014



Higgs et al., 2012

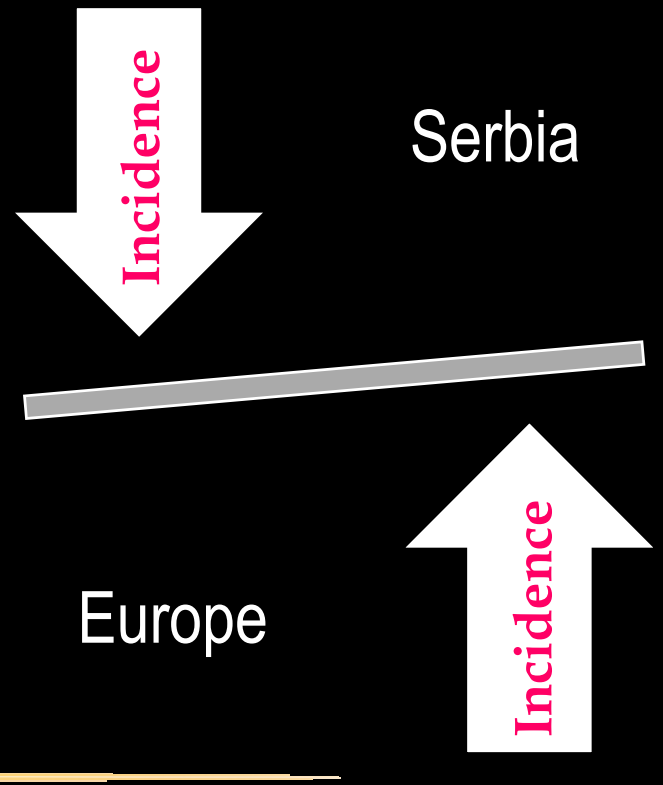
Pertussis incidence in the Republic of Serbia 1965-2011



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Pertussis in Serbia

- Notifiable infectious disease
- Diagnosis - culture and serology



Pertussis vaccine in Serbia

- Vaccination since 1957
- Current vaccine composition - since 1985
- Four vaccine strains:
  - 8/84 (Fim2)
  - 1772/57 and 2047/57 (Fim2,3)
  - 23/81 (Fim3)
- Acellular vaccine can be administred in private practice (last 10 years)
- Acellular vaccine in the imunization calendar from 2014



In Serbia, for more than 50 years, vaccine strains have been changed regularly to coincide with isolates circulating in the susceptible population.

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Study of antigenic divergence *of B. pertussis* in Serbia

- Identification of serotypes and genotypes of *B. pertussis* vaccine strains and circulating isolates between 1953 and 2011.
- Comparision with circulating and vaccine strains in other European countries, USA and Australia.

- **4 vaccine strains:**

  (2047/57, 1772/57, 23/81 and 8/84)

- **77 clinical isolates:**

  I. **1953 to 1960 (n=21)**

  II. **1961 to 1979 (n=9)**

  III. **1980 to 1989 (n=34)**

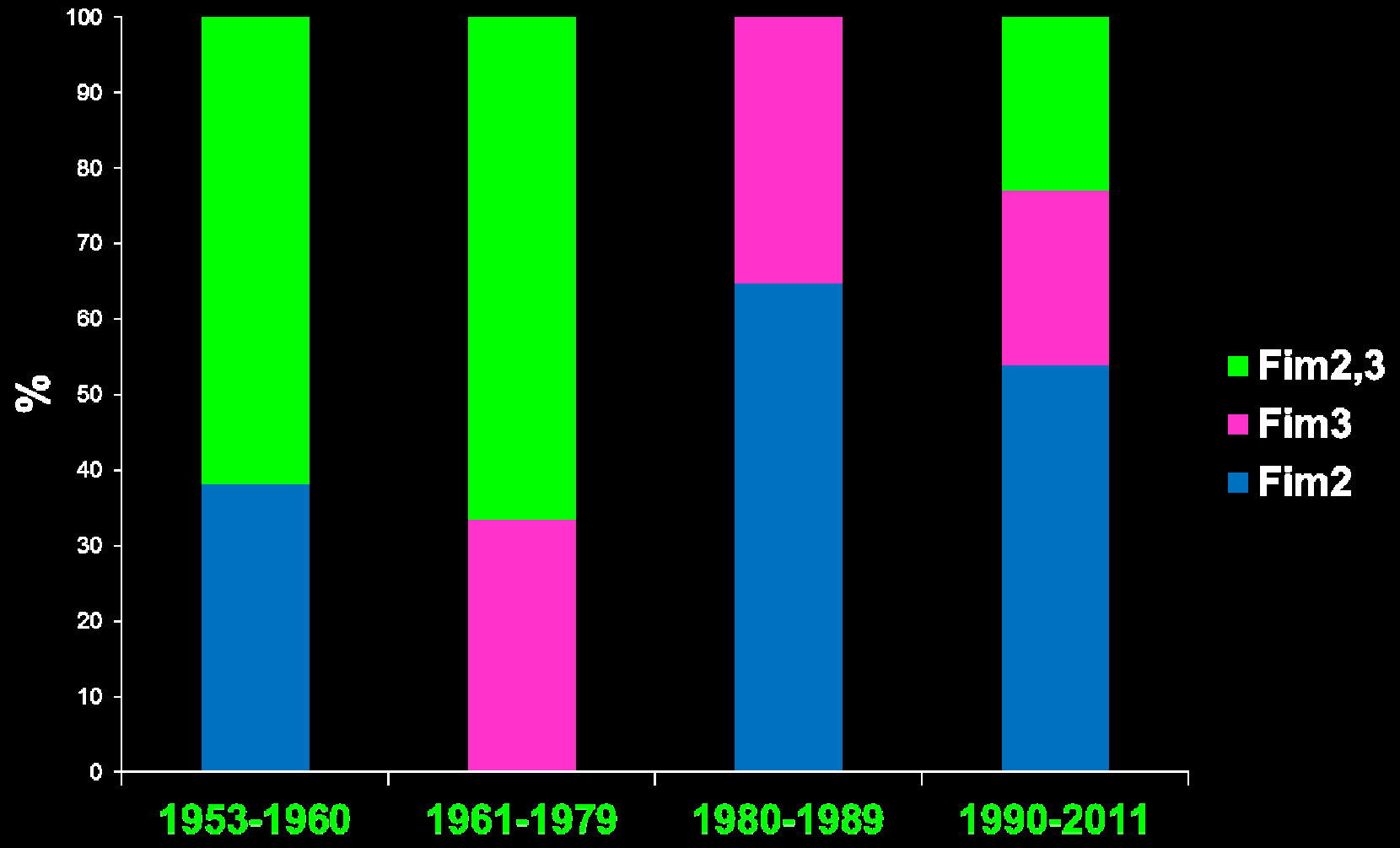
  IV. **1990. to 2011 (n=13)**

- **Serotyping** – monoclonal antibodies against Fim2 and Fim3 by slide agglutination test*
- **Genotyping** - LightCycler PCR & PFGE* :
  - **Ptx S1 subunit(ptxA)**
  - **Prn**
- **PFGE profiles** – pulsed-field gel electrophoresis*

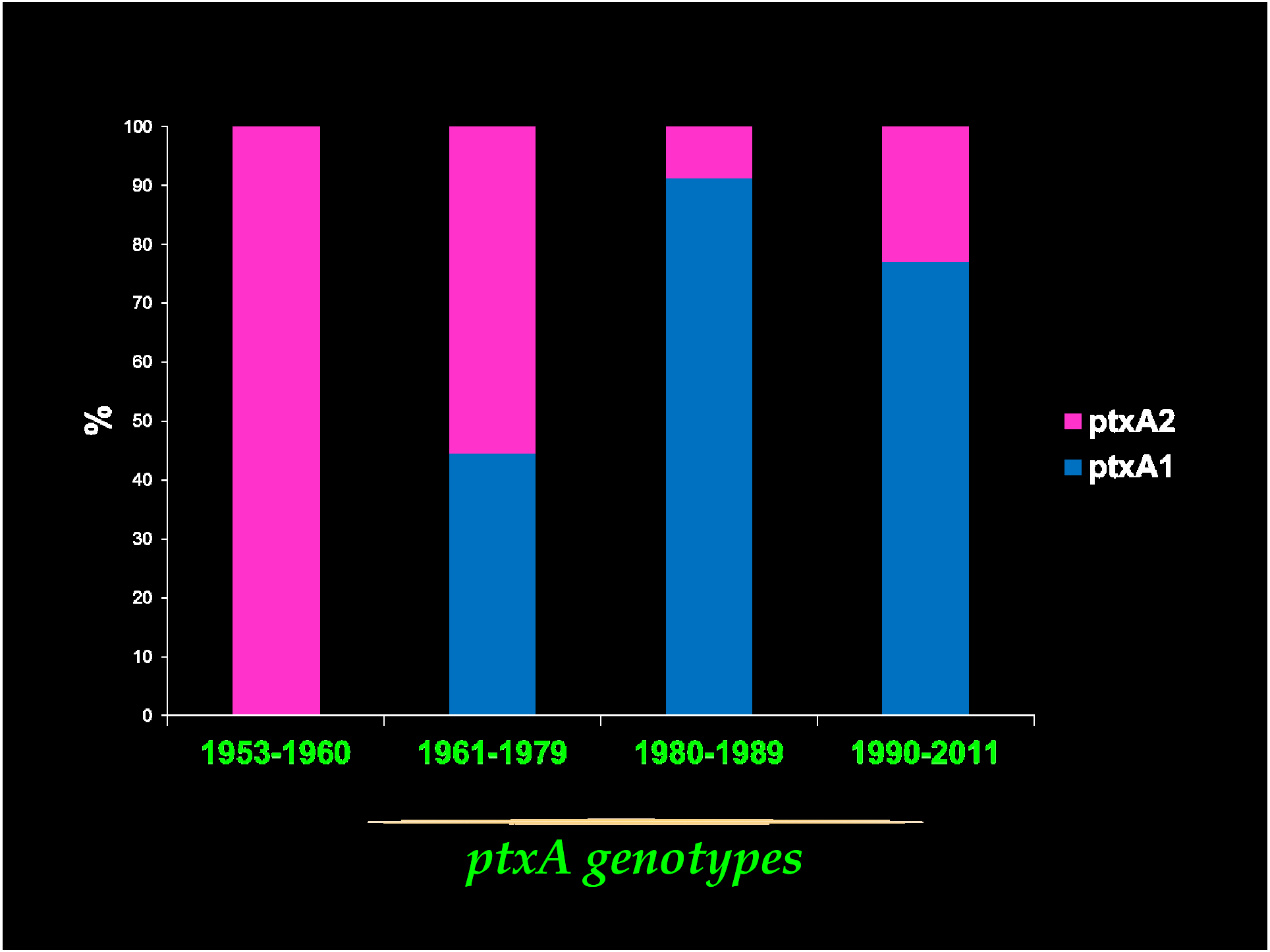
*Advani et al., 2004, Mooi et al., 2000

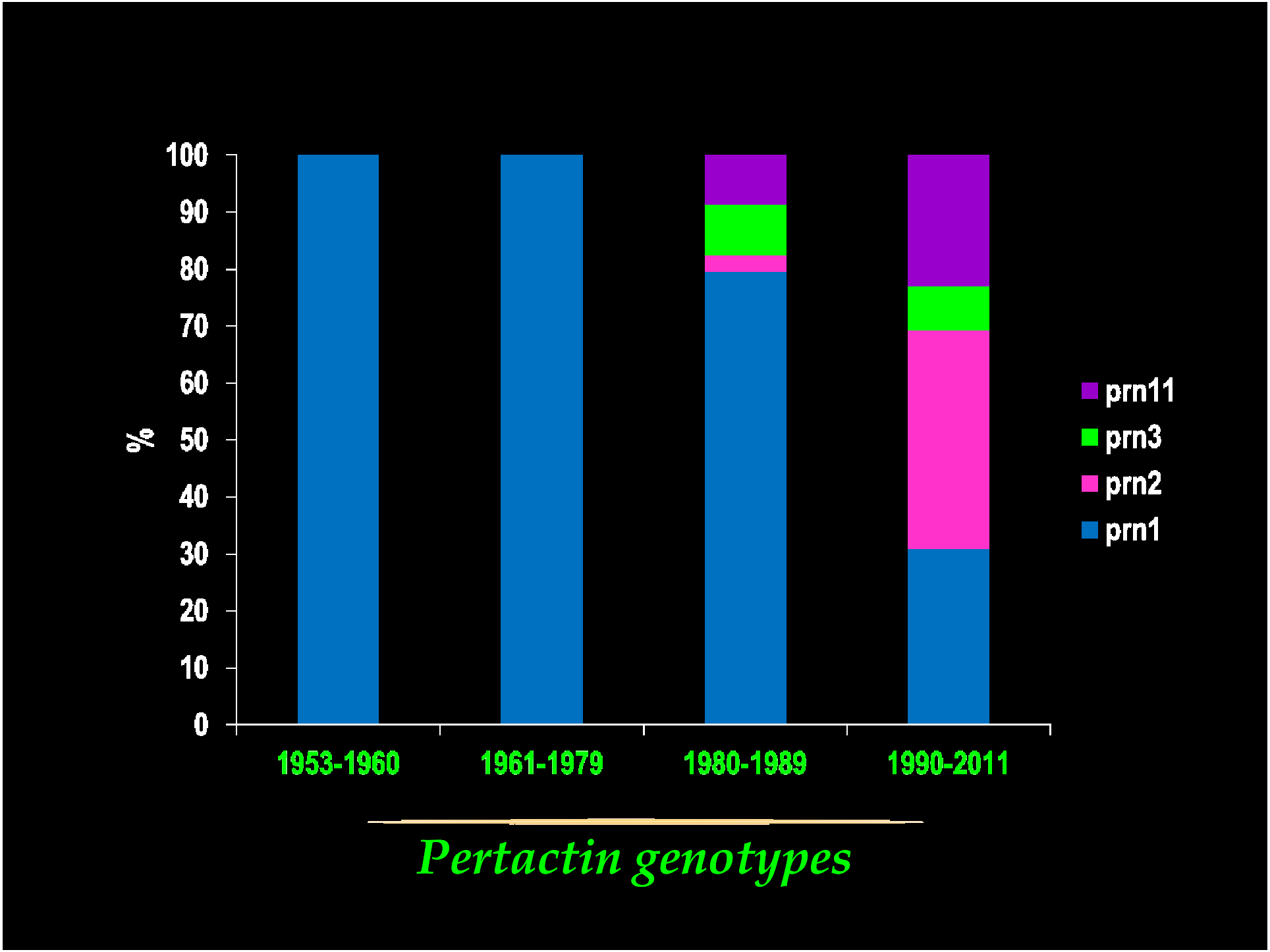
Vaccine strains

Strain	Fim	Prn	PtxA	Isolated	Added in vaccine
1772/57	2,3	1	2	1957	1972
2047/57	2,3	1	2	1957	1968
23/81	3	1	1	1981	1985
8/84	2	2	1	1984	1985



B. pertussis serotypes


100
90
80
70
60
50
40
30
20
10
0
%
1953-1960
1961-1979
1980-1989
1990-2011
ptxA2
ptxA1
ptxA genotypes


100
90
80
70
60
50
40
30
20
10
0
%
1953-1960
1961-1979
1980-1989
1990-2011
prn11
prn3
prn2
prn1
Pertactin genotypes

Summary

Isolation period	No. of isolates	ptxA		prn				serotype		
		ptxA1	ptxA2	prn1	prn2	prn3	prn 11	Fim2	Fim2,3	Fim3
1953.-1960.	21	0	21	21	0	0	0	8	13	0
1961.-1979.	9	4	5	9	0	0	0	0	6	3
1980.-1989.	34	31	3	27	1	3	3	22	0	12
1990.-2011.	13	10	3	4	5	1	3	7	3	3
Summ	77	45	32	61	6	4	6	37	22	18

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	Vaccine strains		Circulating strains	
	Serbia[*1]	Europe[*2]	Serbia[*1]	Europe[*2]
prn	*prn1* and *prn2*	*prn1* or *prn7*	*prn1, prn11*	*prn2, prn3*
ptxA	*ptxA1 and ptxA2*	*ptxA2* or *ptxA4*	*ptxA1*	*ptxA1*

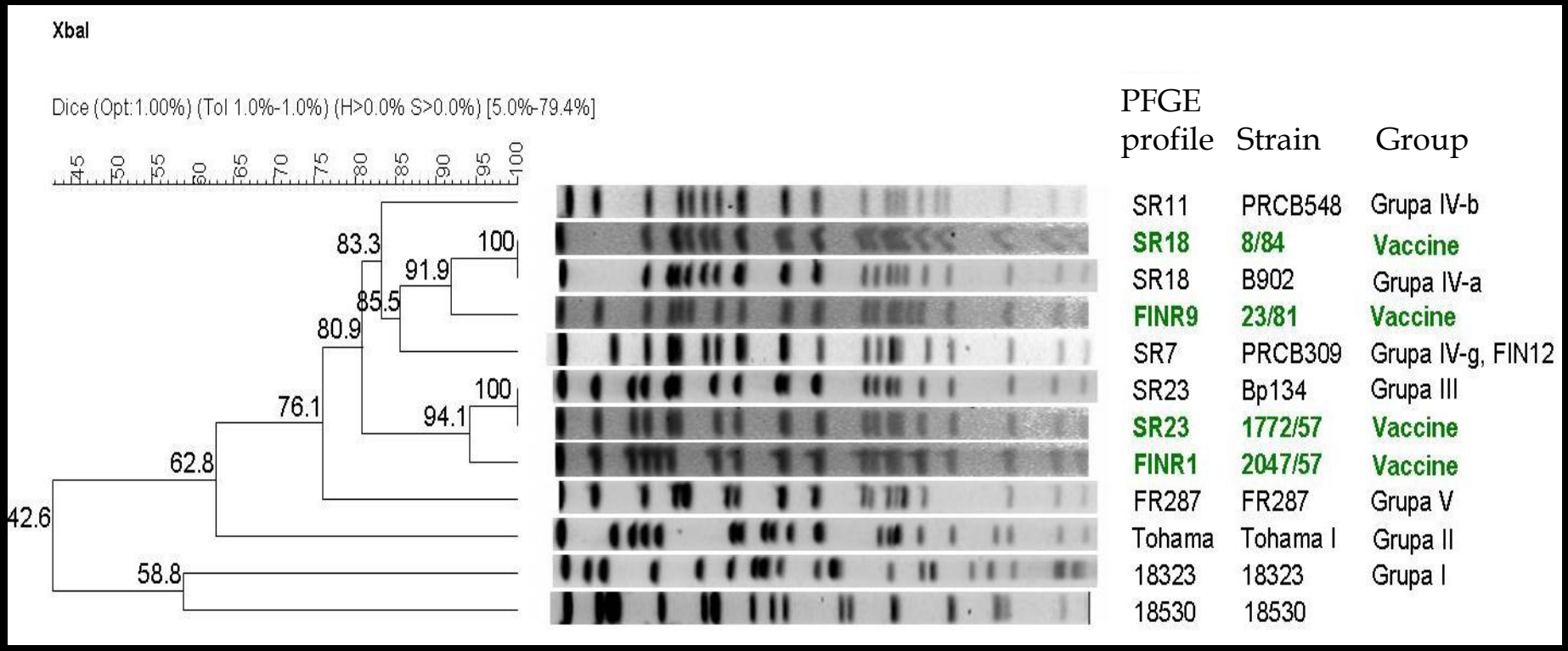
[*1]Dakic et al., 2010.
Pljesa et al., 2014 .

[*2]Mooi et al., 2013
Mooi et al., 2010
He et al., 2009
Litt et al., 2009
Elomaa et al., 2005.

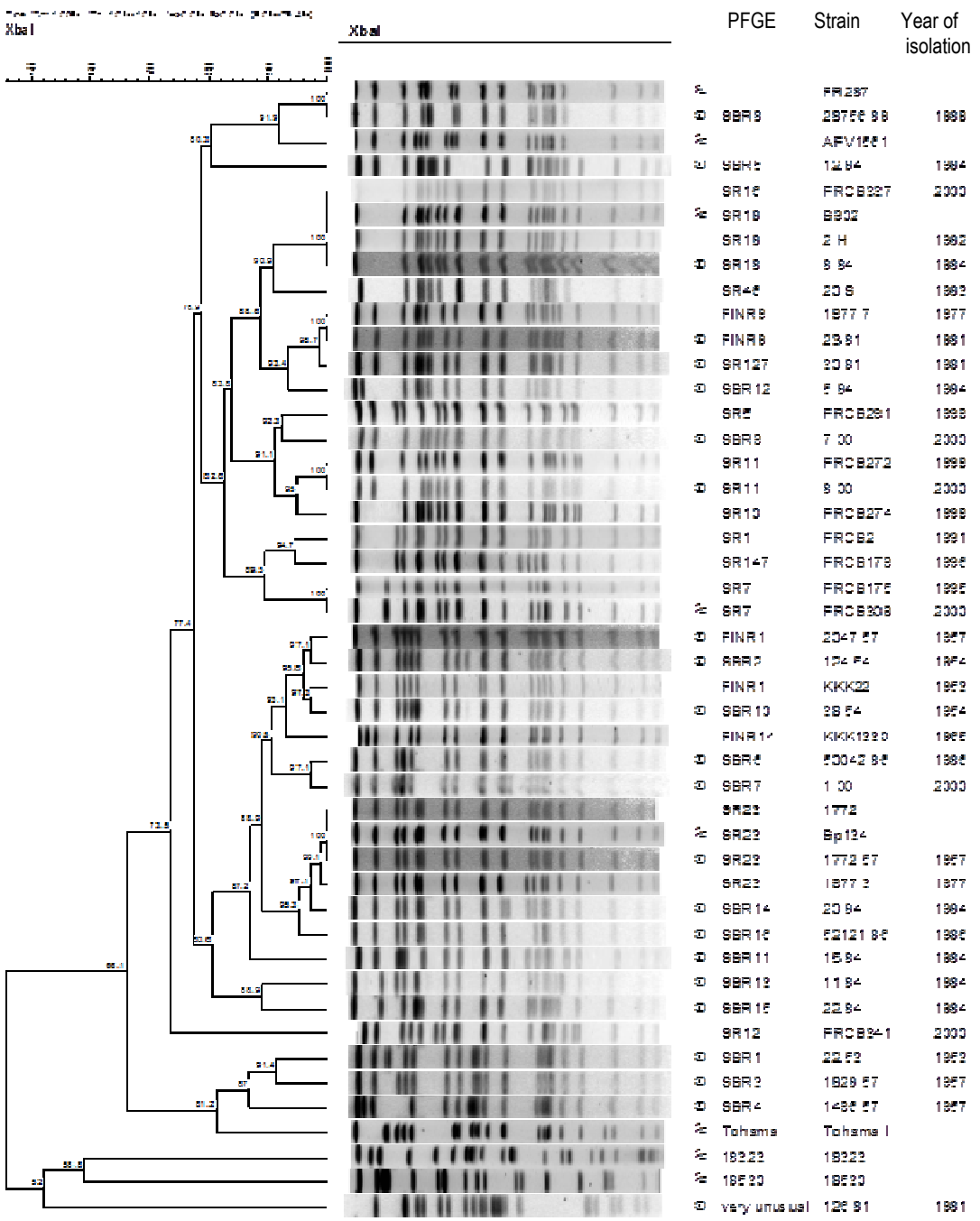
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PFGE profiles of vaccine strains

- **Vaccine strains in Serbia - 4 different PFGE profiles**



PFGE profile	Strain	Group
SR11	PRCB548	Grupa IV-b
SR18	**8/84**	**Vaccine**
SR18	B902	Grupa IV-a
FINR9	**23/81**	**Vaccine**
SR7	PRCB309	Grupa IV-g, FIN12
SR23	Bp134	Grupa III
SR23	**1772/57**	**Vaccine**
FINR1	**2047/57**	**Vaccine**
FR287	FR287	Grupa V
Tohama	Tohama I	Grupa II
18323	18323	Grupa I
18530	18530	

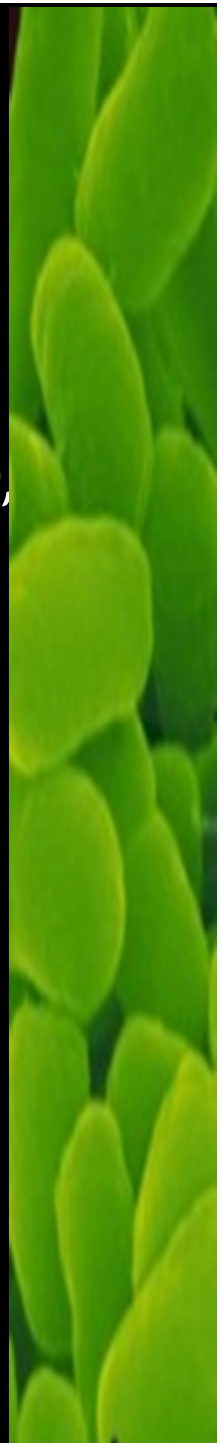


Dendrogram of *B. pertussis* strains

- Isolates - 22 different PFGE profiles
- **43% -** unique Serbian profiles (BpSBR)

PFGE profiles of *B. pertussis* strains

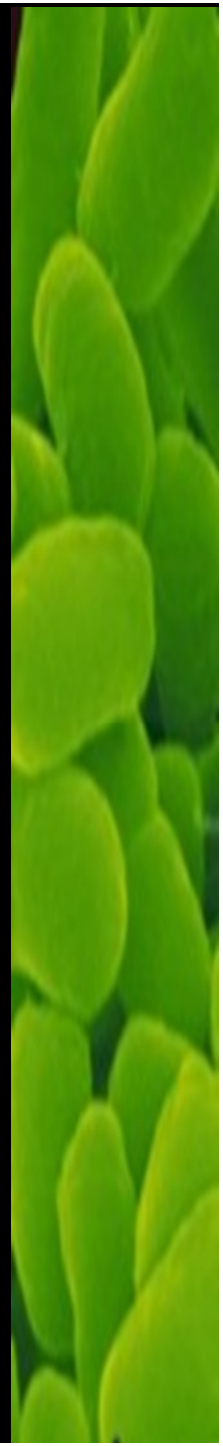
- Change in PFGE profiles was observed over time
- 5 common profiles - 2/3 of isolates (BpSR23, BpFINR1, BpFINR9, BpSBR6 and BpSBR5)
- All PFGE profiles, observed in 1950s disappeared since then (except BpSR23)
- The profile BpSR23 was found in all the study periods
- 95% of isolates belonged to two clusters, having a high similarity with a minimum of 78% overall relatedness



Serbia vs. *other countries*
-Serotyping-

- **Vaccine strains – all 3 serotypes (Fim2, Fim3 & Fim2.3)**
- **After the introduction of vaccination – the frequency of serotype Fim2.3 decreased**
- **Fim2 has been the most prevalent serotype during the study period.**

- ***In most other countries - Fim2 predominate in unvaccinated population & displaced by Fim3 strains when vaccination is introduced (Hallander et al, 2005)***



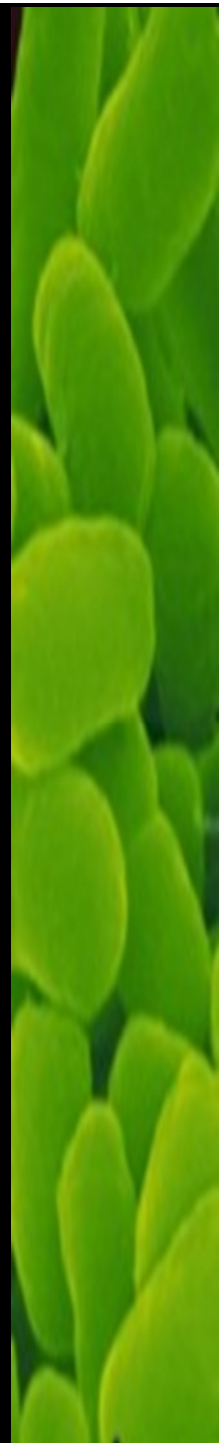
Serbia vs. *other countries*
-Prn genotyping-

- **3 vaccine strains (2047/57, 1772/57 & 23/81) - prn1 and 1 vaccine strain (8/84) - prn2**
- **Isolates - prn1, prn2, prn3 i prn11**
- **Frequency of prn2 genotype – very low and appearance were late (compared to other countries)**
- **Dominant – prn1 and prn11**

- *The prn2 is by far the most prevalent type in modern isolates (Advani et al., 2004; Elomaa et al., 2005; Heikkinen et al., 2008; Mooi et al., 2013)*
- *The prn11 - only in Australia and China, in 1980s (Byrne et al., 2006; Zhang et al., 2010)*

Serbia vs. *other countries*
-Prn genotyping-

- **The low frequency of prn2 strains and their relatively late emergence in Serbia may be due to the fact that the vaccine contains an isolate having prn2 allele (introduced in vaccine composition 1985) !**



Serbia vs. *other countries*
-PtxA genotyping-

- **2 vaccine strains (2047/57 & 1772/57) - ptxA2, 2 vaccine strains (23/81 & 8/84) - ptxA1.**
- **A shift from ptxA2 to ptxA1 has been observed in isolates since the late 1960s**
- **ptxA1 genotype predominant in 1980-1989.**
- **The re-appearance of isolates containing ptxA2 was noticed after the two strains harboring ptxA1 were added into the vaccine in 1985.**

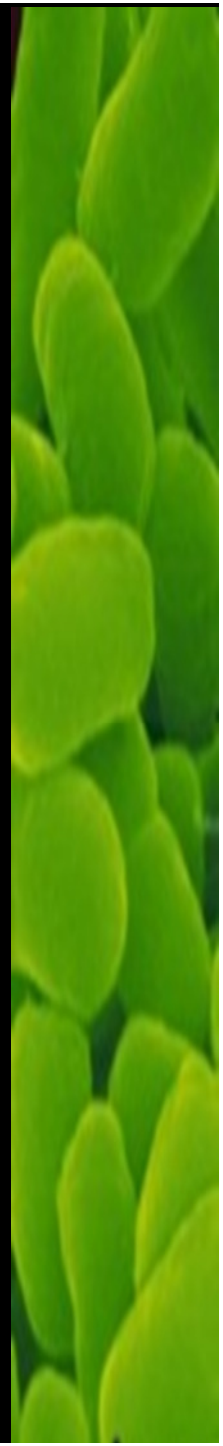
- ***The high frequency of strains harboring ptxA2 in 1990-2011 was not comparable to that noticed in many other countries (Elomaa et al., 2005;Cassiday et al., 2000; Weber et al., 2001)***

Serbia vs. *other countries*
-PFGE analysis-

- **Specific population of circulating *B. pertussis* strains in Serbia**
- **The profile BpSR23, representing 30% of isolates studied, persisted in the whole study period**
- **Only one strain (isolated in 2000) was BpSR11**
- **43% of isolates studied showed unique BpSBR profiles**

- ***BpSR23 was prevalent in Finland and Sweden in 1970s but not after 1990s (Elomaa et al., 2005; Poynten et al., 2004)***
- ***BpSR11 was found to be predominant in six of the eight European countries (Hallander et al., 2007)***

- According to the observed findings, the B. pertussis population in Serbia is different from other vaccinated populations, and this difference may be related to the vaccine composition, that had formulation of inclusion of "contemporary" strains from population.



It has been shown that:

- **variation in Prn affects vaccine efficacy in the mouse model (King et al., 2001)**
- **the adequate bacterial elimination rates were observed in mice immunized and challenged with the same vaccine type strain (Bottero et al., 2007)**
- **the vaccine prepared from a recent isolate provided the highest mouse protection when compared to those prepared from the old isolates such as the strain Tohama I (Pereira et al., 2005).**

- **Increasing evidence that the currently available acellular pertussis vaccines are not providing optimal control of pertussis in the United States and many other countries has stimulated interest in improvements of the current vaccines and in the development of new vaccines**
- **A better understanding of the limitations of the current vaccines and the basis for the pertussis resurgence is needed to design improved vaccines**

Meade et al, 2014.

Modification of antigens in current vaccines:

- **Possible modifications of the current vaccines while maintaining the same antigenic composition include changing of the individual antigens to match antigens of currently circulating stains of *B. pertussis*.**



Meade et al, 2014.

THANK YOU FOR YOUR ATTENTION!

This investigation was performed through collaboration between Institute of Virology, Vaccines and Sera, Torlak, Belgrade and Pertussis Reference Laboratory, National Institute for Health and Welfare (THL) Turku, Finland, courtesy to

Prof.dr Quishe He