GenEpi Webinar

Antigen Surveillance: from Evolution to Immune Escape

A virtual training workshop within GenEpi-BioTrain.

March 23rd, 2026

Carla RODRIGUES & Valérie BOUCHEZ

Intended Learning Objectives

Genomic epidemiology of *B. pertussis*

o *Bordetella pertussis* vaccine strategies (the different types of vaccines, evolution of primary vaccination schedules, adult and pregnant woman vaccination, main focus in France)

o Antigenic variation and partial vaccine escape (evolution of vaccine antigen circulating genotypes, vaccine escape (PRN-negative *B. pertussis* isolates)

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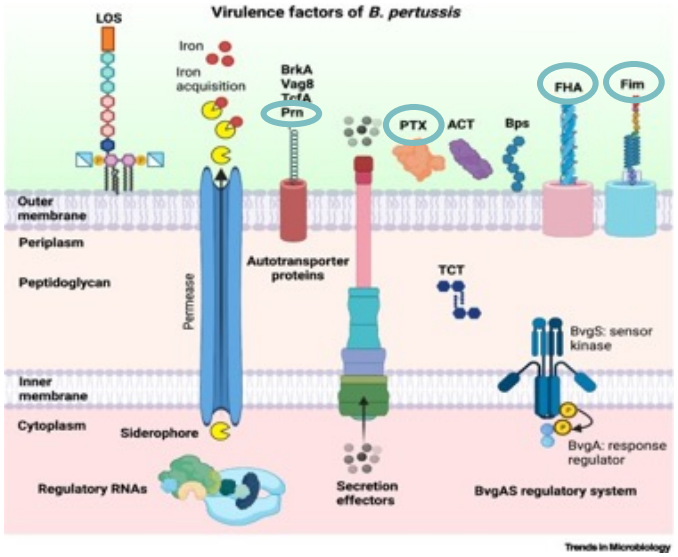
PERTUSSIS : MAIN ASPECTS

Bacterial pathogens: ***Bordetella pertussis*** **(and *B. parapertussis*)**

- Gram-negative coccobacillus (strictly aerobic)
- Slow culture on specific media (Bordet-Gengou, Regan-Lowe)
- Production of virulence factors : Toxins (PT) and Adhesins (FHA, PRN, FIM2 & FIM3)



Mohamed, Yasmine Fathy et al. Trends in Microbiology, Volume 31, Issue 11, 1192 - 1193

Vaccine-preventable disease: whole-cell vaccine replaced by **acellular vaccine** (aPV)

- Contain **2 to 5 vaccine antigens** (PT, FHA, PRN, FIM2 and 3)
- Amount of antigens varies between primary vaccine and boosters

Endemic despite vaccination, **cyclical** (every 3 to 5 years), human-to-human transmission

Very <u>severe disease</u> for infants < 6m and other people at risk

Severe forms ~18% hospitalized in ICU:

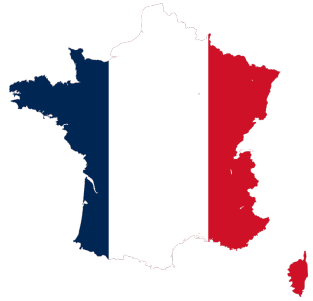
apnea/bradycardia, more rarely **fulminant pertussis**

(Tubiana S, Pediatr Infect Dis J 2015)

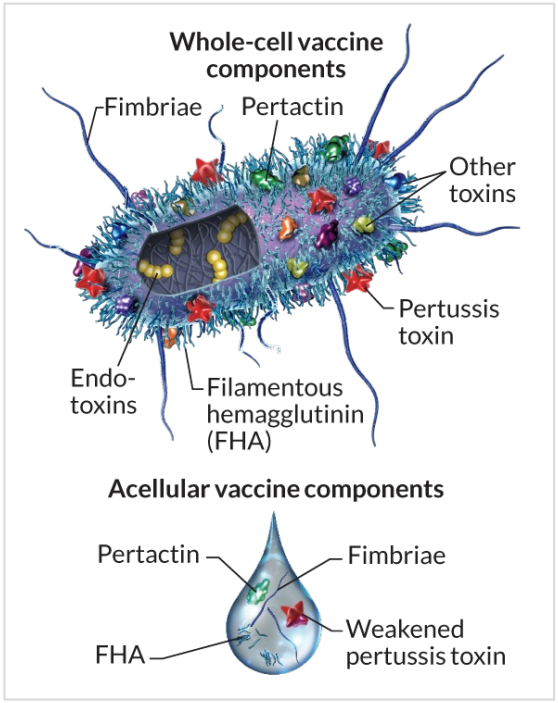
Are you vaccinated against whooping cough?

PERTUSSIS : VACCINATION IN FRANCE

The different types of vaccines



- ✓ **Acellular vaccines** replaced **whole-cell vaccine** in the 1990's in several european countries and in the 2000's in France



Credit: Nicolle Rager Fuller

- ✓ Acellular vaccines contain **2 to 5 vaccine antigens** for primary vaccination and for boosters with different amount of antigens

	Prime vaccination	Boosters
aPV-2 components PT+FHA	Hexyon®	Tetravac®
PT	25 µg	25 µg
FHA	25 µg	25 µg
Marketing Authorisation year	2013	1998
aPV-3 components PT+FHA+PRN	InfanrixHexa® InfanrixQuinta	BoostrixTetra
PT	25 µg	8 µg
FHA	25 µg	8 µg
PRN	8 µg	2.5 µg
Marketing Authorisation year	2000	2005
aPV-5 components PT+PRN+FHA+FIM2+FIM3	Vaxelis®	Repevax®
PT	20 µg	2.5 µg
FHA	20 µg	5 µg
PRN	3 µg	3 µg
FIM2-FIM3	5 µg	5 µg
Marketing Authorisation year	2016	2002

Bouchez et al. 2021 Eurosurveillance, TableS1

PERTUSSIS : VACCINATION IN FRANCE

Vaccination schedules and recommandations

https://vaccination-info-service.fr/Les-maladies-et-leurs-vaccins/Coqueluche

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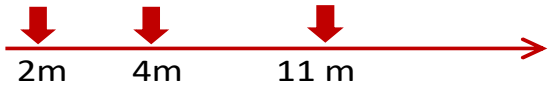
Bouchez et al. 2021 Eurosurveillance, TableS1

Vaccination coverage rate: **>95% at the first booster (SpF)**

Changes in the vaccination schedule

Before April 2013 : schedule 3+1

After april 2013 : schedule 2+1



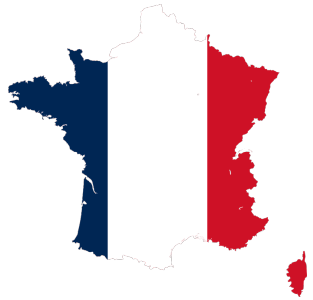
Evolution of the vaccine schedule for adults in 2025

vaccination recommanded every 20 years between 25 and 45 years of age than every 10 years.

Shorter-lived protection induced by the new vaccine schedule →
increase of pertussis cases in children aged 2–5yo

(Paireau *et al.* LID2022)

PERTUSSIS : VACCINATION IN FRANCE



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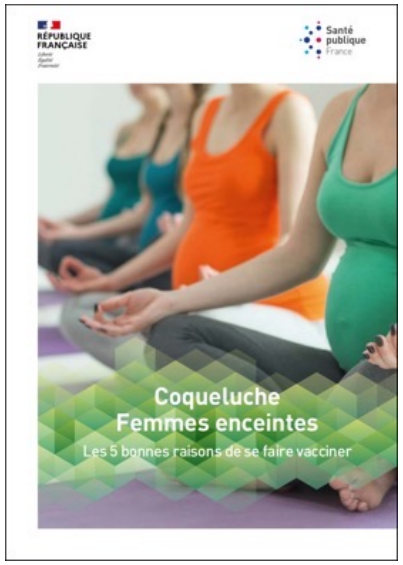
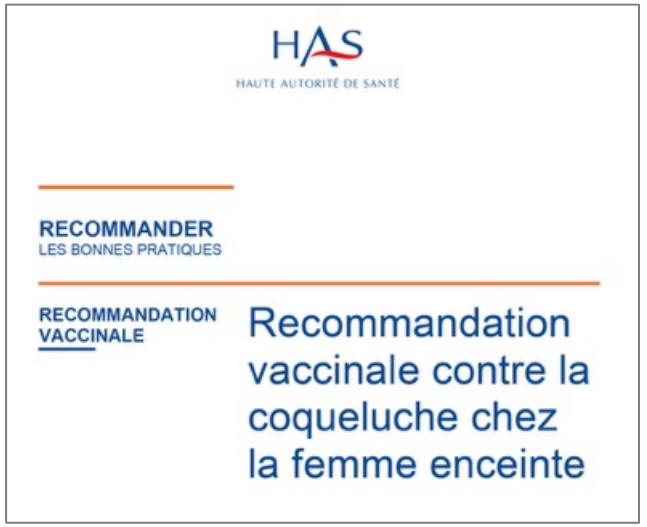


Since April 2022, vaccination against whooping cough is recommended for pregnant women in France

- **During each pregnancy**
- **Between 20 and 36 weeks of pregnancy**


HAS
HAUTE AUTORITÉ DE SANTÉ

RECOMMANDER
LES BONNES PRATIQUES

RECOMMANDATION VACCINALE

Recommandation vaccinale contre la coqueluche chez la femme enceinte

https://www.has-sante.fr/upload/docs/application/pdf/2024-07/recommandation_strategie_de_vaccination_contre_la_coqueluche_dans_le_contexte_epidemique_de_2024_-_r_2024-07-18_14-11-55_706.pdf

- according to HAS the coverage levels - **below 20%** between June 2022 and May 2024
- according to EPI-PHARE study - 2% in 2021, 12% in 2022, 41% in 2023 and **65% in 2024**

INSTITUT Pasteur

Key role of the French National reference center for whooping cough for the surveillance of *B. pertussis* isolates

French hospitals (mainly paediatric) and outpatient laboratories

(1st line diagnostics not carried out at the NRC)

Surveillance/confirmation

Bacterial Strains

Respiratory Samples

DNA

NRC workflow

French hospitals (mainly paediatric) and outpatient laboratories

(1st line diagnostics not carried out at the NRC)

Surveillance/confirmation

Bacterial Strains

- Identification (MALDI-TOF, biochemical tests)
- Antibiogram (disk diffusion, e-test)
- **WGS** (Illumina, Nanopore) for genotyping using BIGSdb and blastN
- **ELISA** => screening of the production of vaccine antigens (PT, FHA, PRN, FIM2 & FIM3)

Respiratory Samples

DNA

qPCR

(combines multiple PCR targets to gain sensitivity and specificity for species ID - Pta, h-IS*1001*, flagellin and a qPCR for detection of the mutation responsible for macrolide resistance)

CULTURING
(Ct IS*481* < 25)

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o Antigenic variation and partial vaccine escape (evolution of vaccine antigen circulating genotypes, vaccine escape (PRN-negative *B. pertussis* isolates)

Focus on *B. pertussis* vaccine antigens

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Bouchez et al. 2021 Eurosurveillance, TableS1

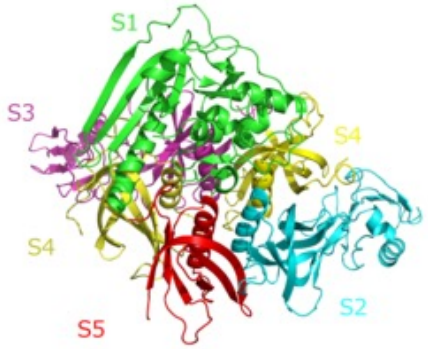
- ✓ **Pertussis toxin (PT)**
- ✓ **Filamentous haemagglutinin (FHA)**
- ✓ **Pertactin (PRN)**
- ✓ **Fimbrial proteins (FIM2 & FIM3)**

What type of vaccines are used in your country?

B. pertussis vaccine antigens

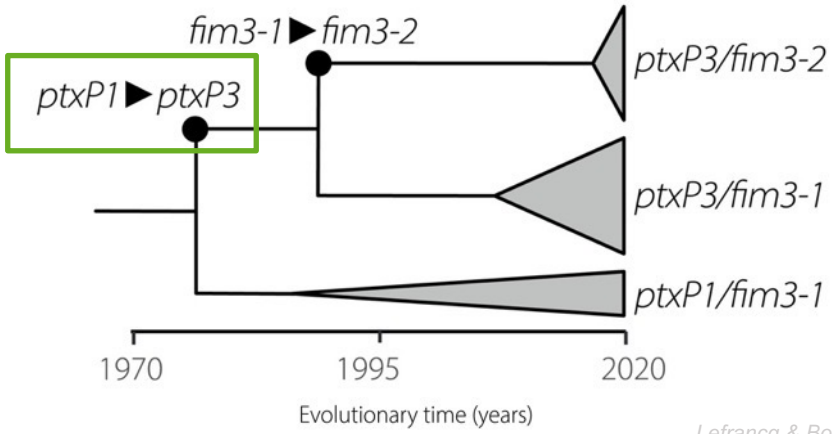
Pertussis toxin (PT)

- a secreted protein
- a complex multi-subunit toxin with an AB5 configuration (subunits S1 to S5)
- enzymatic activity of PT resides in the Subunit S1
- encoded by the *ptx-ptl* operon

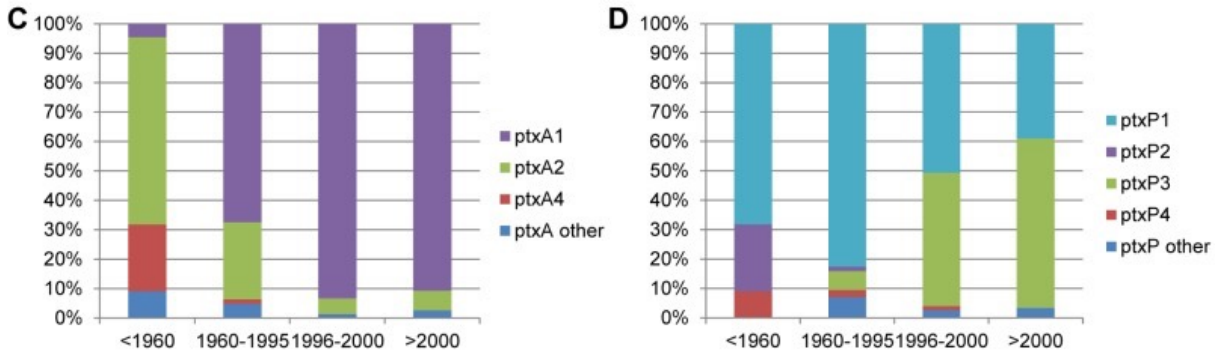


Antigenic variation

- ✓ Polymorphism of the subunit1 (encoded by ***ptx*A** gene) *ptxA*1 replaced *ptxA*2 ones
- ✓ polymorphism of the **promoter of the *ptx-ptl*** operon : ***ptxP*3 isolates replaced *ptxP*1 ones**



Lefrancq & Bouchez et al., STM 2022



Bart et al. Mbio 2014

B. pertussis vaccine antigens

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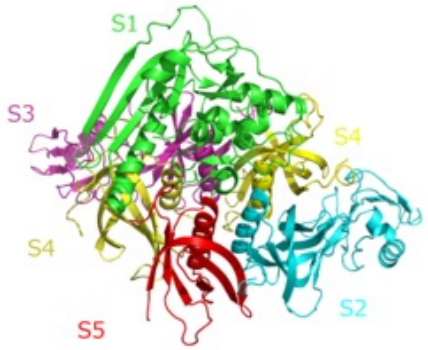

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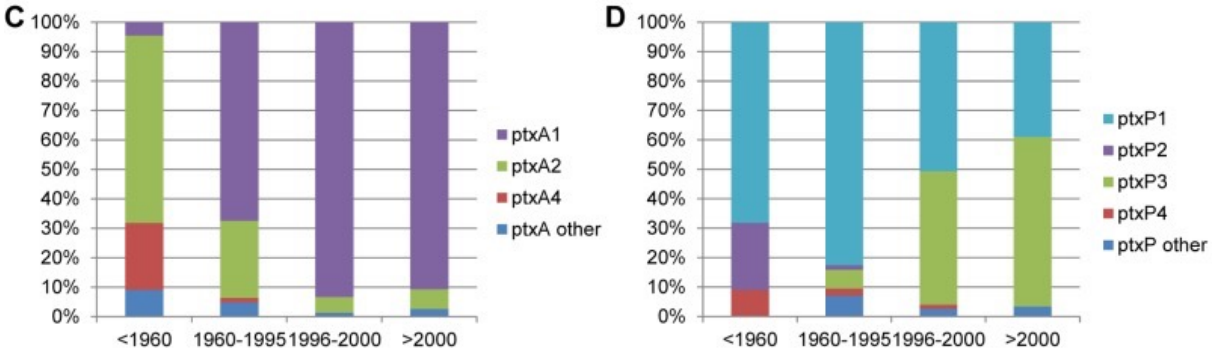
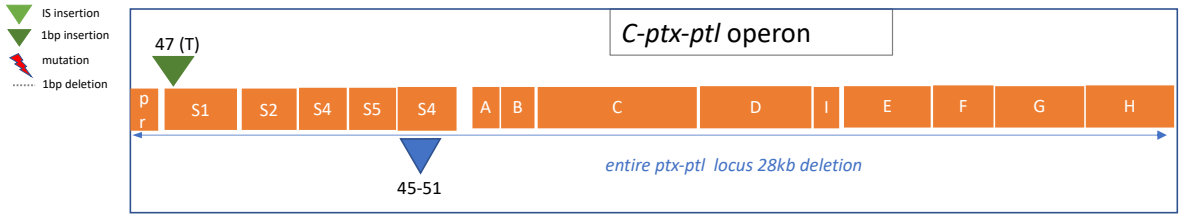


Bart et al. Mbio 2014

PT-negative isolates

- ✓ **PT-negative *B. pertussis isolates* are rare**
- ✓ Genomic events identified for such isolates mainly are insertions and deletions within *ptxP-ptl* operon



Bouchez V et al. Euro Surveill. 2021.

***Bordetella pertussis* Strain Lacking Pertactin and Pertussis Toxin**

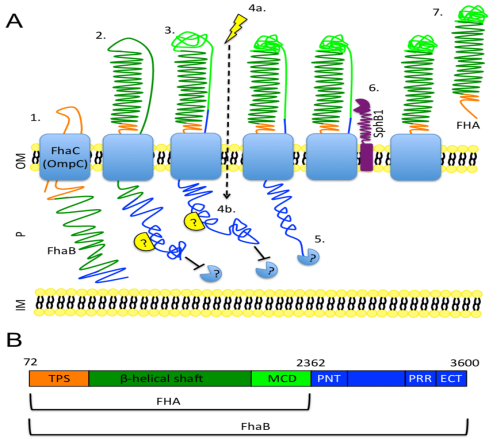
Margaret M. Williams, Kathryn A. Sen, Michael R. Weigand, Tami H. Skoff, Victoria A. Cunningham, Tanya A. Halse, M. Lucia Tondella, CDC Pertussis Working Group[1]

Since the child's birth, the diagnosing HCP had seen the child only once; no visits to other HCPs were known. Per parental report, the case-patient was experiencing paroxysmal cough, apnea, and posttussive vomiting. No thoracic

B. pertussis vaccine antigens

Filamentous haemagglutinin (FHA)

- first synthesized as an ~370-kDa preproprotein called FhaB
- translocated through the FhaC barrel
- cleaved by sphB1 protease
- surface associated but also secreted



Antigenic variation

- ✓ a specific allele a *fhaB* gene has been found for some macrolide resistant isolates (called *fhaB*3 or *fhaB*39)
- ✓ This allele is associated to *ptxP1* genotype circulating in China (Fu et al., 2024)

B. pertussis vaccine antigens

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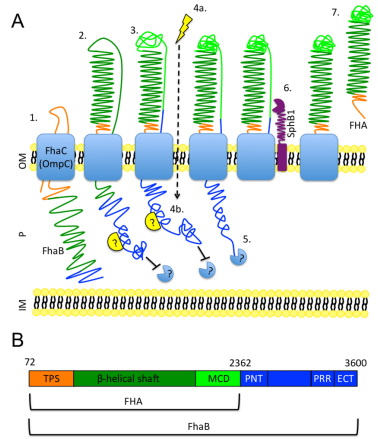

Emergence of Erythromycin-Resistant and Pertactin- and Filamentous Hemagglutinin-Deficient *Bordetella pertussis* Strains — Beijing, China, 2022–2023

Guilan Zhou[1,&]; Ying Li[2,&]; Hairui Wang[1]; Yuanyuan Wang[2]; Yuan Gao[1]; Juan Xu[1]; Fengshuang Wang[2]; Tao Peng[2]; Maojun Zhang[1]; Zhujun Shao[1,#]

Iran J Public Health, Vol. 50, No.7, Jul 2021, pp.1454-1462

Original Article

Fha Deficient *Bordetella pertussis* Isolates in Iran with 50 Years Whole Cell Pertussis Vaccination

*Samaneh Saedi [1], Azadeh Safarchi [1], Faranak Tayebzadeh Moghadam [1], Siamak Heidarzadeh [2], Vajihe Sadat Nikbin [1], *Fereshteh Shahcheraghi [3]*

1. *Department of Bacteriology, Pasteur Institute of Iran, Tehran, Iran*
2. *Department of Microbiology and Virology, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran*
3. *Pertussis Reference Laboratory, Department of Bacteriology, Pasteur Institute of Iran, Tehran, Iran*

FHA-negative isolates

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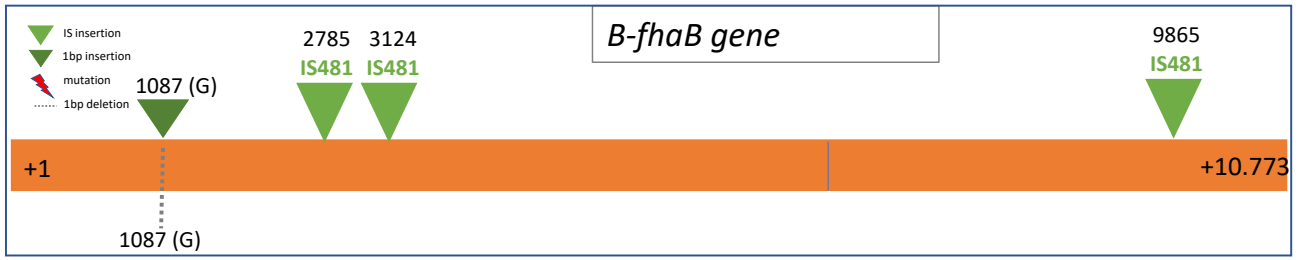
- ✓ Genomic events identified in such isolates mainly are insertions and deletions.

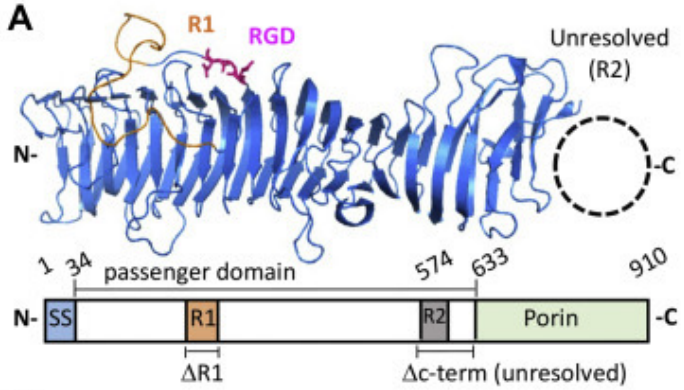


Bouchez V et al. Euro Surveill. 2021.

B. pertussis vaccine antigens

Pertactin (PRN)

- 69 kda autotransporter
- membrane protein
- highly immunogenic
- the immunodominant and immunoprotective epitopes of pertactin include two repeated regions, I and II.



Antigenic variation

✓ A Polymorphism of Repeated Regions of Pertactin protein was identified in *Bordetella pertussis*, *Bordetella parapertussis*, and *Bordetella bronchiseptica* *(Boursaux-Eude & Guiso, Inf.Imm., 2000)*

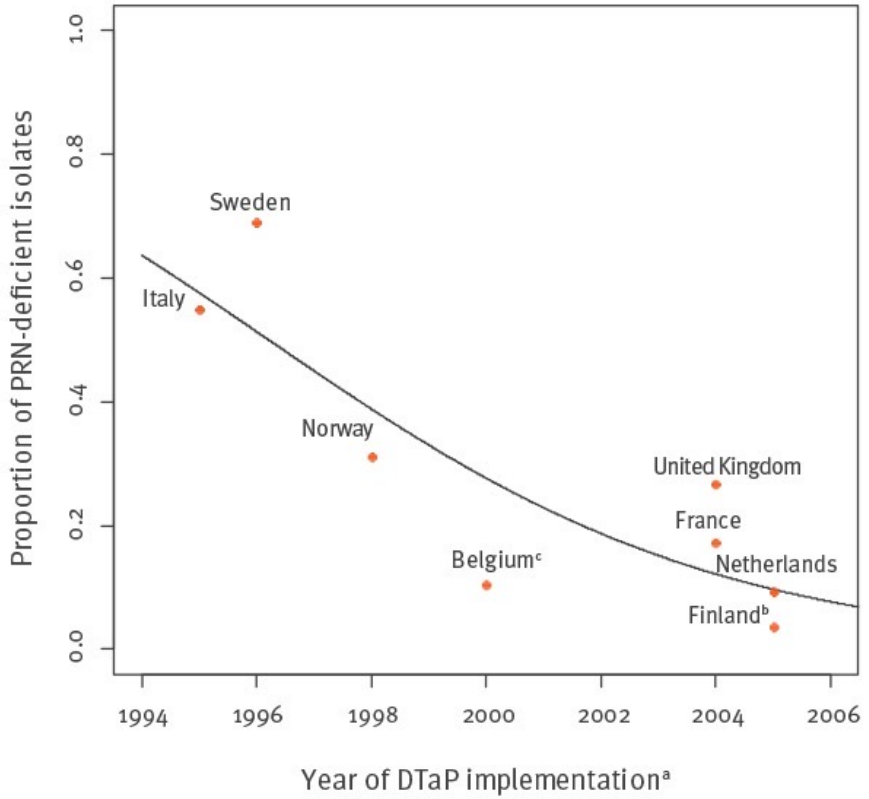
- Bp vaccine strains produce a **PRN 1** (encoded by ***prn*1** allele)
- Bp recent isolates produce a **PRN 2** (encoded by ***prn*2** allele)
- Recent macrolide resistant emerging strains from China are characterized by allele ***prn*150** (C531T synonymous mutation compared to allele *prn*2)

	R1	R2
Prn1	$(GGAVP)_3$ $(GGFGP)_2$	$(PQPG)_1$ $(PQP)_3$
Prn2	$(GGAVP)_2$ $(GGFGP)_4$	$(PQPG)_1$ $(PQP)_3$
Prn3	$(GGAVP)_2$ $(GGFGP)_3$	$(PQPG)_1$ $(PQP)_3$
***Bbr* Prn**	$(GGAVP)_2$ $(GGFGP)_1$	$(PQPG)_2$ $(PQP)_4$
***Bpp* Prn**	$(GGAVP)_3$ $(GGFGP)_1$	$(PQPG)_2$ $(PQP)_6$

B. pertussis vaccine antigens

Pertactin (PRN) >> PRN-deficient isolates

- ✓ **PRN-deficient *B. pertussis* isolates** appeared and increased in frequency after 2007 in countries using acellular vaccines
- ✓ The frequency of PRN-deficient strains varied according to countries : the United States (85%), Australia (>80%), Sweden (69%), Italy (55%), France (55%)
- ✓ A correlation was found between **the introduction of a primary Acellular pertussis vaccine** containing pertactin (PRN) in a European country and the **proportion of PRN-deficient isolates**

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Research Open Access

Pertactin-deficient *Bordetella pertussis* isolates: evidence of increased circulation in Europe, 1998 to 2015

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Check for updates

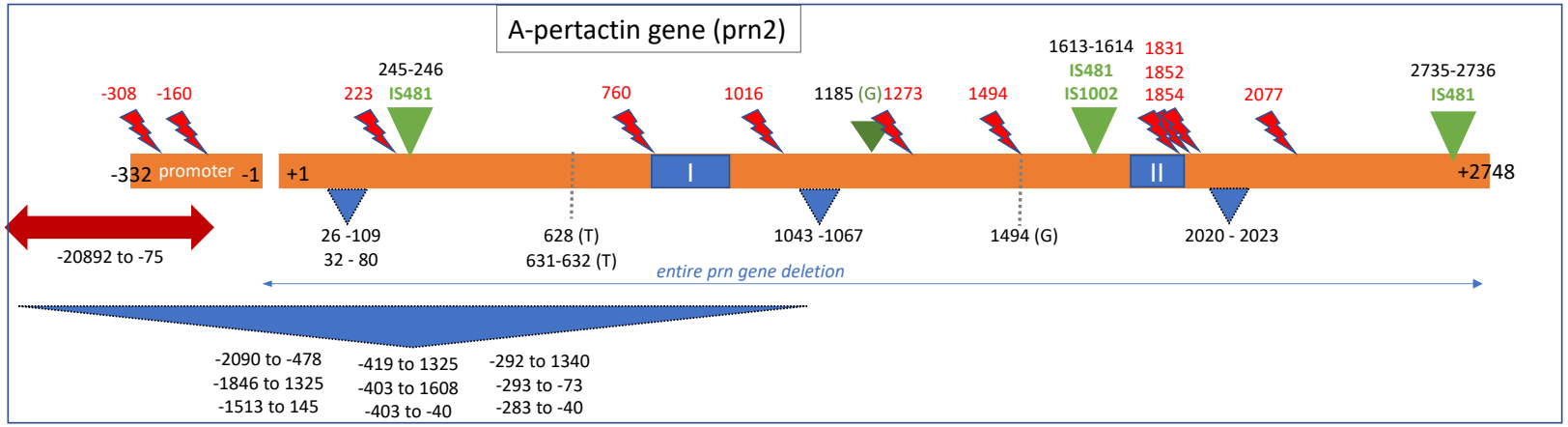
Alex-Mikael Barkoff[1], Jussi Mertsola[2], Denis Pierard[3], Tine Dalby[4], Silje Vermedal Hoegh[5], Sophie Guillot[6], Paola Stefanelli[7], Marjolein van Gent[8], Guy Berbers[8], Didrik Vestrheim[9], Margrethe Greve-Isdahl[5], Lena Wehlin[10], Margaretha Ljungman[10], Norman K. Fry[11], Kevin Markey[12], Qiushui He[1,13]

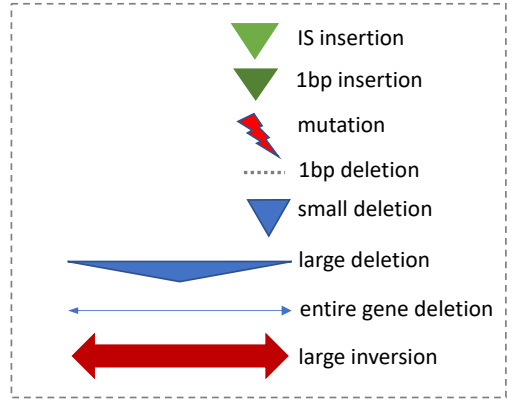
Barkoff et al., Eurosurv. 2019
Ma et al., EID., 2021
Bouchez et al., Eurosurv. 2022

B. pertussis vaccine antigens

Pertactin (PRN) >> PRN-deficient isolates

- ✓ The PRN-deficient isolates are **genetically diverse,** as different mutations can inactivate the *prn* gene (IS insertion, deletions, mutations, inversions. The more frequently observed are : IS481 insertion and a large inversion.



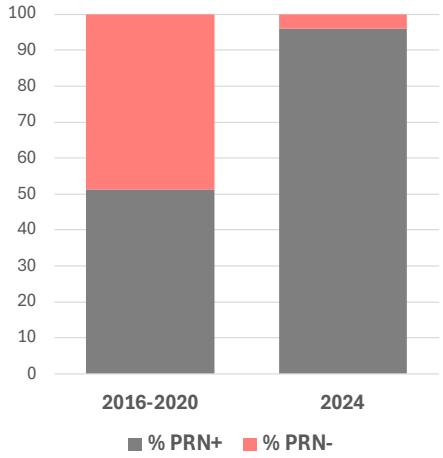


Bouchez V et al. Euro Surveill. 2021.

B. pertussis vaccine antigens

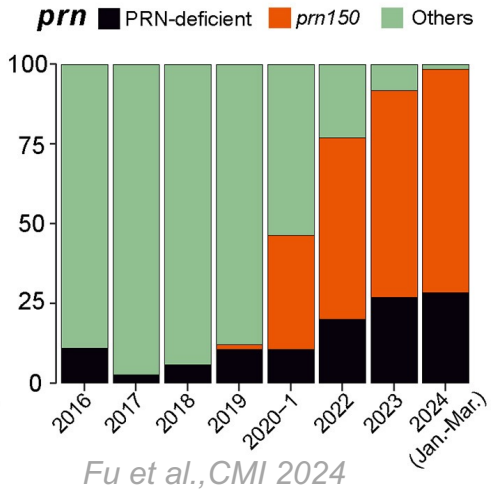
Pertactin (PRN) >> PRN-deficient isolates

- After COVID_19 pandemic, the circulation of *B. pertussis* highly decreased in all countries.
  - In Europe, post-pandemic *B. pertussis* isolates were mostly **producing PRN** (*prn2* allele)



Rodrigues et al. Eurosurveillance, 2024
Data from France

  - In Asia, post-pandemic *B. pertussis* isolates were mostly resistant to macrolide, some producing PRN (allele *prn150*) and others being **PRN-deficient.**

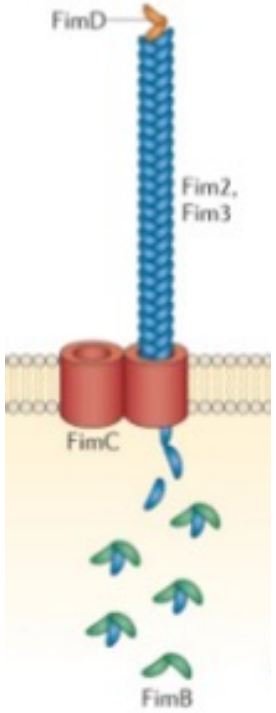


Fu et al.,CMI 2024
Data from Shanghai

B. pertussis vaccine antigens

Fimbriae proteins FIM2 & FIM3

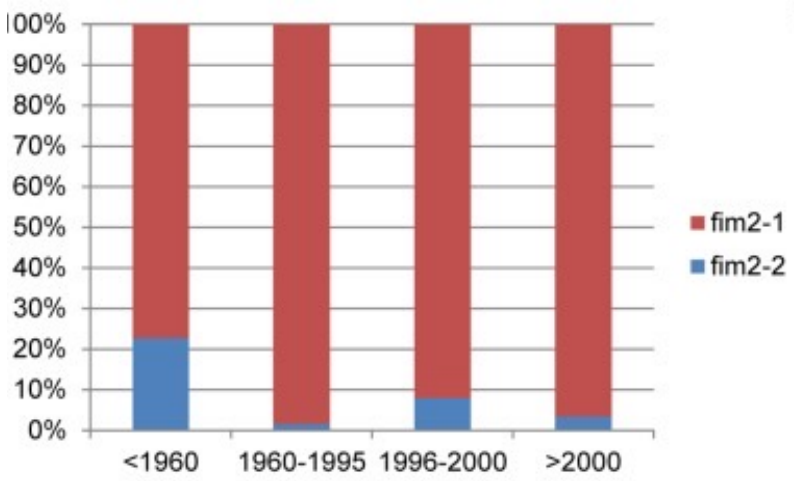
- 2 distinst proteins
- *B. pertussis* isolates can produce FIM2, FIM3, both or none



Antigenic variation

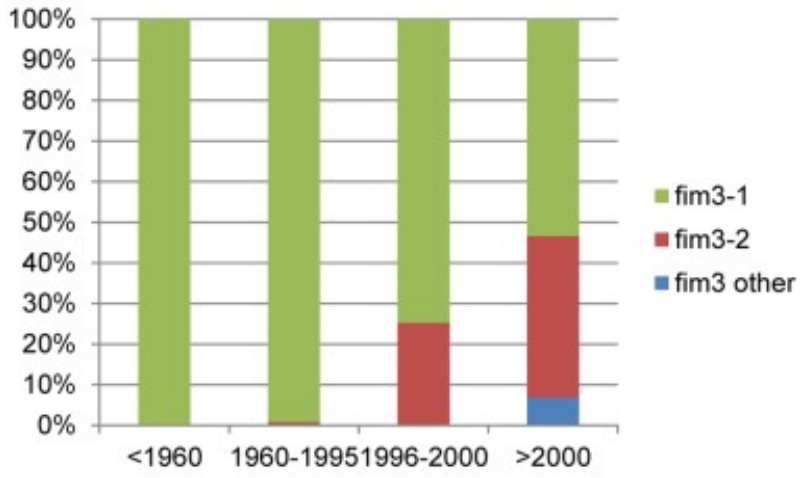
Polymorphism of *fim2* gene

- ✓ 2 main alleles *fim2-1* and *fim2-2*
- ✓ recent isolates all display *fim2-1*



Polymorphism of *fim3* gene

- ✓ 2 main alleles *fim3-1* and *fim3-2*
- ✓ recent isolates display *fim3-1* or *fim3-2*



Bart et al. Mbio, 2014

Zoom on the evolution of the population structure of *B. pertussis*

- ✓ in nearly all countries, the proportion of *ptxP3* isolates increased from 5 to 20% in the early 1990s to >80% by mid-2000s
- ✓ By contrast, *fim3* allele distribution has been more steady over the years
- ✓ **Increased fitness of pertactin-deficient strains after implementation of acellular vaccines**, but reduced fitness otherwise

- ✓ based on 3344 whole-genome sequences
- ✓ from 23 countries
- ✓ over an 85-year period (1935-2020).

SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

MOLECULAR EPIDEMIOLOGY

Global spatial dynamics and vaccine-induced fitness changes of *Bordetella pertussis*

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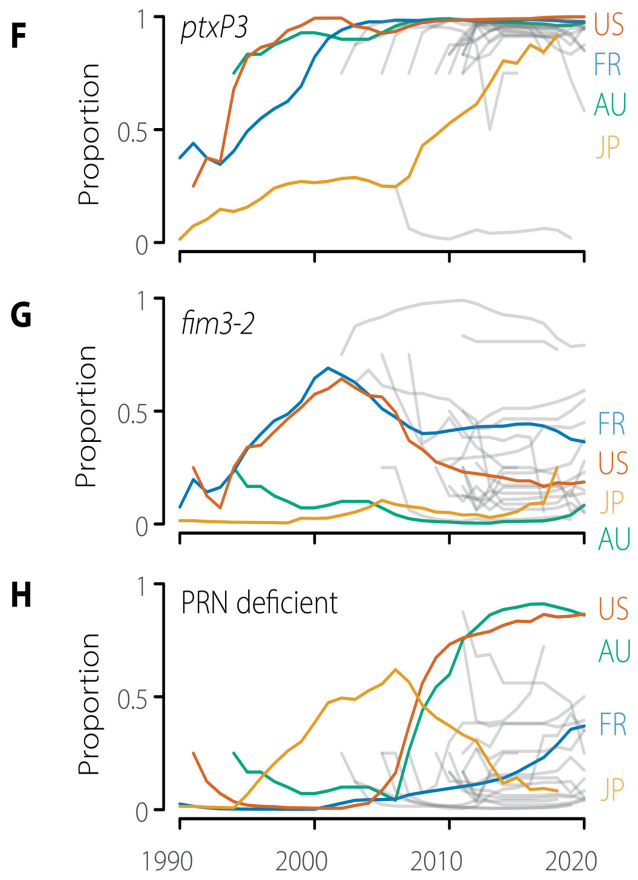

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CONCLUSIONS

- ✓ Surveillance of the evolution of the **production of *B. pertussis* vaccine antigens** (PT, FHA, PRN, FIM2&FIM3) is important in order to confirm the adequation of current acellular vaccines.
- ✓ Surveillance of the **genomic evolution of the vaccine antigens** is also important and contributes to the global genomic surveillance of *B. pertussis* under vaccine pressure.
- ✓ While diagnosis of whooping cough is based on qPCR, the characterization of *B. pertussis* isolates by National reference centers is therefore essential and relies for the moment on the culture of the bacteria.
- ✓ Metagenomic **culture-independent sequencing methods** are currently being developed by different teams and will allow to improve this surveillance in the future.
  (Luu et al. JCM 2024; Peng et al., JCM 2024; Fong et al. The Lancet 2026)

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Practical Session:

Pertussis vaccine antigen detection

Valérie Bouchez

GenEpi Webinar :

Antigen Surveillance: from Evolution to Immune Escape

A virtual training workshop within GenEpi-BioTrain.

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