A novel and versatile immune assay to distinguish and monitor vaccination and infection-induced antibody-mediated recognition of *Bordetella pertussis*

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Major differences exist between whole-cell (wP) and acellular (aP) pertussis vaccines with respect to their antigenic content. Currently, most antibody assays for pertussis are focused on individual vaccine antigens. We therefore established a flow cytometry-based assay to quantify IgG and IgA binding to whole *B. pertussis* (Bp) in the various PERISCOPE clinical studies. This assay allows us to answer whether and how (sub)clinical infections shape the immune response to *B. pertussis* (Bp), particularly in aP-primed populations. To distinguish infection- from aP-induced antibodies, we constructed a mutant Bp strain (Bp_mut) that lacks the aP antigens FHA, Prn and PT (Fig 1a). Next, we quantified antibody binding to Bp_mut in in nasal mucosal lining fluid (MLF) samples collected from 7-10y old children (n=32) and 11-14y old adolescents (n=30) who were primed with aP during infancy. Adolescents showed significantly higher baseline antibody binding to Bp_mut than children (Fig 1b), suggesting increased exposure to Bp in older aP-primed individuals. These findings were in line with pertussis disease surveillance data (Fig 1c). Interestingly, high baseline antibody binding to Bp_mut strongly correlated with enhanced persistence of mucosal antibodies following subsequent Tdap-IPV vaccination, suggesting that differences in Bp exposure between birth cohorts may influence the immune response to vaccination. Currently, we are investigating mucosal antibody binding to Bp wild type and Bp_mut in MLF samples collected from infants following maternal vaccination and primary vaccination with wP or aP. Moreover, we aim to further evaluate circulation of Bp in a large Dutch cross-sectional cohort study.

Figure 1. The use of antigen-deficient B. pertussis to detect infection-induced antibodies. a. A mutant B. pertussis strain was constructed that does not express the aP vaccine antigens PT, PRN and FHA. b. aP-primed adolescents (cohort B, 11-14y) show significantly higher baseline mucosal IgG binding to Bp_mut than aP-primed children (cohort A, 7-10y). Antibody binding is shown as log2 mean fluorescent intensity. c. Cumulative pertussis disease incidence based on regional pertussis notifications in both birth cohorts, from birth until study inclusion.
Validation of ELITe InGenius® and Bordetella ELITe MGB® Kit for the molecular diagnosis of Bordetella pertussis and related species

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The clinical laboratory of microbiology of the UZ Brussel, which acts as the Belgian National Reference Centre (NRC) for Bordetella pertussis together with the Sciensano Service of Immunology, currently utilises an in-house developed qPCR assay for the detection and identification of Bordetella pertussis, B. parapertussis and B. holmesii in respiratory samples (Martini et al., 2017).

We evaluated the CE-IVD-labelled ELITe InGenius® device, together with the Bordetella ELITe MGB Kit® (ELITechGroup) as an RIVD-approved alternative to the in-house assay. Both assays are based on two screening targets: IS481 and IS1001; as well as two confirmation targets: recA and IS1002 (in-house assay) or ptxA-Pr (ELITe assay).

The ELITe InGenius®, an automated benchtop instrument, enables sample-to-result processing, as it is capable of nucleic acid extraction, real-time PCR and result interpretation in a single run. This limits the hands-on work time.

In our validation efforts, accuracy was evaluated by performing the assay on a selection of 85 respiratory samples, previously tested with the in-house PCR. Both methods showed full concordance with regard to the sample result after interpretation.

A limited verification of the sensitivity confirmed or surpassed the limits of detection claimed by ELITechGroup. This verification was done for all three species, using negative nasopharyngeal aspirates spiked with a known concentration of a reference strain or recent clinical strain.

Both intra-run and inter-run precision sufficed, with coefficients of variation not surpassing 3% for any of the targets or species.

Two runs were performed with alternating high-concentration samples and negative controls in order to check for cross-contamination. None was detected.

Finally, a selection of Bordetella spp. different from the three target species was tested in order to assess specificity. Most showed no cross-reactivity, minor cross-reactions were observed for B. bronchialis and B. bronchiseptica, these results were concordant with those of the in-house PCR. Significant cross-reactivity was observed for B. petrii, this is currently being investigated further.

In conclusion, the ELITe InGenius® and Bordetella ELITe MGB Kit® showed satisfactory results in terms of accuracy, sensitivity, precision and specificity, for the detection of B. pertussis, B. parapertussis and B. holmesii in respiratory samples.
First results from an External Quality Assessment scheme targeting the Antimicrobial Susceptibility Testing among the European Pertussis Reference Laboratories

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Background
Laboratory confirmation of pertussis is important for treatment, prevention and surveillance of the disease. Conventionally, macrolides (erythromycin and azithromycin) are the first line drugs for (prophylactic) treatment. However, the appearance of *Bordetella pertussis* isolates, resistant to macrolides, have been notified in several countries including France, the US and China. Furthermore, a dramatic increase of these isolates occurred in 2013 in China, and currently up to 90% of the *B. pertussis* isolates were found to be macrolide resistant in this country. So far, all studies have shown that the molecular mechanism of *B. pertussis* resistance to macrolides is associated with an A2047G mutation in the 23S rRNA gene. Therefore, molecular tests targeting a specific gene of interest, by single gene sequencing, allelic specific PCR or whole genome sequencing (WGS), are important. To evaluate this capability, external quality assurance (EQA) study is highly needed.

Aim
We aimed to evaluate the capability of European national pertussis reference laboratories (NRL) to identify macrolide resistant or sensitive *B. pertussis* isolates.

Methods
This study was organised by the European Reference Laboratory Network for Pertussis (ERLNPert-Net) consortium under the ECDC framework contract (ECDC/2019/023). Invitation to participate in the EQA study was sent to 30 European NRLs. Altogether, 17 NRLs confirmed to participate in the study. The study panel consisted of 11 coded/blinded DNA samples, together with both positive and negative controls. The panel samples included DNAs extracted from macrolide sensitive or resistant *B. pertussis* isolates in varied concentrations. The participating laboratories were asked to use their own in-house methods to analyse the EQA samples and report the results.

Results and conclusion
This is an ongoing study. The deadline for result submission is in August and complete result analysis will be performed and presented during the meeting.
The first external quality assurance scheme for *Bordetella pertussis* vaccine antigen expression

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Background

The non-expression of vaccine antigen targets in circulating *Bordetella pertussis* isolates is a cause for concern. The ability to detect both vaccine antigen expression and lack of expression in *B. pertussis* isolates is important for pertussis surveillance activities. External Quality Assessment (EQA) is a critical component in assessing laboratory performance. As part of the Coordination of the European Reference Laboratory Network for Pertussis (ERLN-Pert-Net), we delivered the first EQA scheme for *B. pertussis* vaccine antigen expression: fimbriae (FIM), pertactin (PRN), pertussis toxin (PT) and filamentous haemagglutinin (FHA), among the National Reference Laboratories in EU/EEA countries.

Methods

The test panel included eight strains of *B. pertussis* together with three control strains. The EQA was divided into three sections: (i) FIM serotyping, (ii) PRN/PT expression and (iii) FHA expression. Laboratories were asked to complete as many sections as they were able to.

Results

Twelve laboratories participated in EQA scheme. Eleven completed FIM serotyping; ten completed PRN, six PT and seven FHA expression (or prediction). Only 2 of 11 (18%) laboratories scored 8/8 (100%, intended results) in section (i) fimbrial serotyping. All 11 laboratories used monoclonal antibodies (Mabs). Analysis was performed by ELISA for 5/11 and slide agglutination by 6/11 laboratories. Eight of 10 (80%) laboratories scored 8/8 (100%, intended results) in section (ii) for the ability to distinguish PRN expression/non-expression in *B. pertussis*. Most laboratories (8/10) used the recommended ELISA protocol, with five using the PeM4 pertactin Mabs, two the Non-WHO Reference Material *B. pertussis* polyclonal anti-69kD serum 97/558 (NIBSC) and one, in-house pertactin polyclonal antibodies. Four of six (67%) laboratories scored 8/8 (100%, intended results) in section (ii) for the ability to distinguish PT expression/non-expression. Three of these laboratories used the recommended ELISA protocol with the anti-pertussis toxin monoclonal antibodies 99/512 (S1) and 99/542 (S3) (NIBSC) and one used Western blot with an in-house polyclonal pertussis toxin antibody. Six of seven (86%) laboratories scored 100%, in section (iii) for the ability to distinguish FHA expression/non-expression, four using the recommended ELISA protocol with the Non-WHO Reference Material Anti-Filamentous Haemagglutinin Monoclonal Antibody 2E9 (99/572, NIBSC), one using Western blot with an in-house polyclonal FHA antibody and one using whole genome sequencing (WGS) to predict FHA expression. Three laboratories used analyses from WGS to predict expression, two for PRN, one for PT and two for FHA. One laboratory scored 7/8 for PRN, PT and FHA; one laboratory scored 8/8 for PRN and the other 8/8 for FHA.

Conclusions

Overall results for the first EQA on vaccine antigen (PRN, PT, FHA) expression were encouraging especially for pertactin which was the priority target due to the apparent worldwide rise in circulating PRN non-expressing strains. Training needs in fimbrial serotyping and vaccine antigen expression were identified. Explanations for low overall scores for fimbrial serotyping include potential variation in phenotypic expression due to delay in transport of the panel and differing pre-test culture conditions. In some instances WGS and analysis successfully identified known mutations and previously described reasons for non-expression, but in other cases the phenotypic prediction was incorrect.

Pertussis national reference laboratories should be able to perform fimbrial serotyping and vaccine antigen expression assays for *B. pertussis* isolates. EQAs such as these serve to help ensure comparability of results between laboratories and identify areas for improvement.
An unexpected outbreak with *Bordetella parapertussis* on a paediatric ward: lessons learned

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In 2021, an unexpected increase in positive *Bordetella parapertussis* tests was seen among paediatric patients in a Dutch teaching hospital. This resulted in enhanced infection control and microbiological surveillance among potential contacts (patients, family members and healthcare workers), as well as national public health notifications. Contact tracing investigations revealed that 72% of the contacts tested positive for *B. parapertussis* by PCR; a suspicious outcome.

In this presentation, we will discuss how the collaborative efforts of the laboratory, clinic and public health officers resolved the outbreak by tracing the positive results to contaminated eSwabs. We will briefly review what we do, and do not, know about *B. parapertussis* and laboratory diagnosis thereof.
Anti-\textit{Bordetella pertussis} bactericidal antibody responses to natural infection or vaccines in infants, children and adults

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Analysis of pertussis vaccine antibody responses has focussed for many years on quantification of antibody binding to a limited range of antigens. Antibody Fc-mediated engagement of human complement proteins is likely to be important for opsonophagocytosis and bactericidal killing of \textit{Bordetella pertussis} (Bp). Whilst this has been studied using blood samples, complement proteins can be measured in nasal lining fluid at concentrations sufficient to allow killing of Bp with bactericidal antibodies so this may also cause clearance of Bp on the mucosal surface. We have developed a killing serum bactericidal assay (SBA) where Bp is incubated with heat-inactivated serum and IgG and IgM-depleted human plasma, incubated for 2h and surviving bacteria determined by viable count. We have also developed a duplexed flow cytometry assay to quantify antibody-mediated complement C3b and C5b-9 deposition (ADCD) onto Bp. We have analysed sera from several clinical studies in mothers vaccinated in pregnancy, infants from vaccinated mothers and following booster vaccination in subjects aged 7-10, 11-15, 20-34, 60-70 years. Sera from individuals following pertussis disease or following administration of a live attenuated pertussis vaccine were also analysed.

SBA and ADCD following aP in all ages correlated strongly with anti-Prn IgG concentration. Natural infection, wP and live attenuated vaccines induced SBA against both WT (Prn+) and Prn- strains. Geomean SBA titres obtained with adult sera following aP were similar across all studies except for adults 60-70-year-old who had a lower geomean SBA response. Vaccination in pregnancy provided strong cross-placental transfer of bactericidal antibodies. Higher SBA titres were seen in infants before the first infant dose at 2 months than following two or three doses of aP. This indicates that vaccination in pregnancy provides highly functional antibodies to the infants for their first weeks following birth.
Effectiveness of pertussis vaccination in pregnancy to prevent hospitalisation in infants aged <2 months and effectiveness of both primary vaccination and mother’s vaccination in pregnancy in infants aged 2-11 months


Background
PERTINENT is an active hospital-based surveillance system for pertussis in infants. In 2019, four of the six participating European countries recommended pertussis vaccination in pregnancy to protect infants too young for vaccination. Among infants aged less <2 months, we measured the effectiveness of vaccination in pregnancy; among infants aged 2-11 months, the effectiveness of vaccination in pregnancy and of primary vaccination (PV).

Methods
From December 2015 to December 2019, we included all infants aged <1 year presenting with pertussis-like symptoms from 14 hospitals. Using a test-negative-design, cases were infants testing positive for *Bordetella pertussis* by PCR or culture. Controls were those testing negative for all *Bordetella* species. Vaccinated mothers were those who received pertussis vaccine in pregnancy. Vaccinated infants were those who received at least one dose of pertussis PV >14 days before symptom onset. We excluded infants too young for developing pertussis symptoms (<4 days), with unknown maternal or PV status or with mothers vaccinated within 14 days of delivery. We calculated pooled vaccine effectiveness (VE) as 100*(1-odds ratio of vaccination) adjusted for study site, onset date in quarters and infants’ age group.

Results
Of 829 infants presenting with pertussis-like symptoms, 336 (41%) were too young for PV. For the analysis of VE in pregnancy, we included 75 cases and 201 controls. Vaccination in pregnancy was recorded for 9 cases (12%) and 92 controls (46%), adjusted VE was estimated between 75% [95%CI: 35-91%] and 88% [95%CI: 57-96%].

Among the 493 infants eligible for PV (aged 2-11 months), we included 123 cases and 253 controls. Thirty-one cases and 98 controls recorded both PV with at least one dose and vaccination in pregnancy, adjusted VE was estimated between 74% [95%CI: 33-90] and 95% [95%CI: 69-99]; 27 cases and 53 controls recorded PV only, adjusted VE was estimated between 68% [95%CI: 27-86] and 94% [95%CI: 59-99].

Conclusion
Our findings suggest that vaccination in pregnancy reduces pertussis incidence in infants too young for PV. In infants aged 2-11 months, PV only and both PV and vaccination in pregnancy provide significant protection against severe pertussis.
An update on the novel, live attenuated pertussis vaccine BPZE1

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The main shortcomings of current acellular pertussis vaccine include their failure to prevent *Bordetella pertussis* infection and transmission, the short-lived immunity they induce and the emergence and spread of vaccine-escape mutants. In contrast to vaccination, natural infection by *B. pertussis* prevents subsequent re-infection, provides long-lived immunity and induces immune responses to a broad range of antigens, thereby limiting the emergence of vaccine-escape mutants. In order to mimic natural infection without causing disease, we therefore developed a live attenuated pertussis vaccine for nasal administration. The vaccine strain, named BPZE1, was attenuated by the genetic inactivation or removal of three major *B. pertussis* toxins: pertussis toxin, dermonecrotic toxin and tracheal cytotoxin. In pre-clinical studies the vaccine was shown to be safe, including in severely immuno-compromised mice, and able to induce strong protection, both in mice and non-human primates. BPZE1 is now in advanced stage clinical development and has successfully completed four clinical trials. In a first-in-human study including 12 participants per group, a low dose of $10^3$, a medium dose of $10^4$ and a high dose of $10^7$ colony-forming units (CFU) were compared to placebo for safety, colonization and immunogenicity. Frequency and severeness of adverse events were equal among all groups and the vaccine was shown to be able to colonize the human respiratory tract transiently and to induce antibodies against *B. pertussis* antigens. However, even at the highest dose tested, only 5/12 participants were colonized and induced specific antibodies. Interestingly, the non-colonized subjects had high levels of pre-existing antibodies to *B. pertussis* antigens, suggestion recent exposure to *B. pertussis*. In the next, dose-finding study, subjects with high levels of pre-existing antibodies were excluded, and doses of $10^7$, $10^8$ and $10^9$ CFU were compared to placebo. This study demonstrated safety up to the highest dose and showed sero-conversion in 12/12 participants at the highest dose. The $10^9$ CFU dose was also tested in small group of subjects with high base-line levels of anti-*B. pertussis* antibodies, which resulted in sero-conversion by 4/6 participants. This dose was therefore chosen for further clinical development. First, a bridging study was performed, comparing a liquid formulation administered as nasal drops to a reconstituted lyophilized formulation administered as a nasal spray. Both formulations were equally safe and the reconstituted lyophilized formulation administered by a spray devise appeared to induce the strongest mucosal IgA response. This formulation was used in a subsequent phase 2b study, confirming safety and immunogenicity at the mucosal and systemic levels. In addition, a second dose of BPZE1 given as an attenuated challenge showed that BPZE1 prevented colonization by a second dose, providing the first proof of concept that BPZE1 may protect against *B. pertussis* colonization. Additional studies are currently under way to study safety and immunogenicity in adolescents and to provide proof of concept for protection against virulent *B. pertussis* in a controlled human infection model.
An update on maternal vaccination in England: what's polio got to do with it?

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Background
In England, acellular pertussis-containing vaccine is offered to infants at 8, 12, 16 weeks, followed by a pre-school booster at 3 years and 4 months. Since October 2012, acellular dTaP-IPV vaccine has been offered in every pregnancy. England’s third trimester maternal pertussis vaccination programme was revised to a second trimester recommendation in 2016.

Methods
National laboratory-confirmed pertussis cases were followed up to determine vaccination history, maternal vaccination history and hospitalisation. Pertussis hospital admissions were extracted from the Hospital Episodes Statistics dataset. Vaccine effectiveness (VE) was calculated for laboratory-confirmed cases born between October 2012 and September 2018 using the screening method and matching with a nationally representative dataset.

Results
Laboratory-confirmed pertussis cases peaked during the 2011/2012 outbreak when disease levels were their highest in 20 years. Disease rates overall remained elevated post-2012 with a cyclical peak in 2016/2017 and the beginning of a cyclical peak in 2019/20. From April 2020, following SARS-CoV-2 control measures, pertussis cases fell across all age groups with case numbers the lowest in the last decade.

Higher coverage was observed after earlier maternal vaccination recommendations with approximately 40% of pregnant women vaccinated ≥13 weeks before delivery. Cases and hospitalisations remained elevated in those aged ≥6 months but not in younger infants. No deaths have arisen in babies with vaccinated mothers after earlier maternal vaccination timing and estimates of vaccine effectiveness of the maternal programme against disease and death in <3 month infants remains high with no evidence of a blunted response to the primary course.

Discussion
High levels of protection against pertussis disease in young infants has been retained following a change to the maternal programme offering vaccination from 16 weeks gestation.

The UK undertakes routine environmental surveillance for polio viruses as part of the WHO polio eradication programme. Since February 2022 Vaccine-like type-2 poliovirus has persistently been isolated from sewage samples in Northeast London with indications of person-to-person community transmission. Vaccine coverage is well below the national average in London. There is also evidence of a reduced response to the polio component of primary vaccinations after maternal vaccination. The Joint Committee on Vaccination and Immunisation has therefore recommended urgent catch-up and a supplementary booster campaign in London targeting 1-9-year-olds in London with IPV-containing vaccine.
**Bordetella pertussis** vaccine-induced fitness changes

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**Background**

Asymptomatic carriage and co-circulating lineages hide the underlying dynamics of *Bordetella pertussis* (*Bp*) from surveillance systems. Therefore, strain fitness at the population level remains largely unknown, as does the role of vaccines in driving potential changes. The increasing availability of *Bp* whole-genome sequences can help.

**Materials**

Here we use a unique dataset of 3344 whole-genome sequences representing 23 countries, including newly sequenced isolates from France and 12 European countries (n=1332; 1994-2018). We develop an analytical framework that quantifies the relative fitness of each genotype by vaccination era (whole-cell and acellular vaccine eras), fitting data from all countries in parallel. We assume that the relative fitness of the different genotypes is the same across countries but allow for a change in fitness following the vaccine switch, which occurred at different times in different countries.

**Results**

We find that some *Bp* genotypes are significantly fitter than others, e.g. pertactin-deficient isolates are on average 1.1 times more fit than their pertactin-producing counterparts. Further, we find that implementation of acellular vaccines is linked with large-scale changes in fitness and can explain long-term genotype dynamics. For instance, we find that the vaccine switch is associated with an increased fitness of PRN-deficient genotypes, but only in isolates with a *ptxP3* background, with the effect further increased for *fim3-L* genotypes. On average, PRN-deficient isolates are 1.3 times as fit as PRN-positive isolates after the introduction of the acellular vaccine.

**Conclusions**

These novel insights into *Bp* dynamics and its interactions with vaccine-induced immunity are highly relevant to vaccination policies.
Estimating the spatial dynamics of *Bordetella pertussis*, from local transmission to global dissemination

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**Background**

*Bordetella pertussis* (Bp), which causes whooping cough, infects >24 million individuals annually despite widespread vaccination. Asymptomatic carriage and multiple circulating lineages hide the underlying dynamics of Bp from surveillance systems. Therefore, the extent of spread across spatial scales remains poorly known. Models informed by pathogen sequences can help.

**Aim**

We characterise the diversity of Bp across different spatial scales (within-district, within-country, intra continental and global) and assess the importance of the local population size in driving the diversity of circulating lineages.

**Methods**

We use 3344 whole-genomes sequences representing 23 countries from 6 continents, including 1012 newly sequenced isolates from France and 320 newly sequenced genomes from 12 European countries from the EUpertStrain consortium. Genome-wide single nucleotide polymorphism variation was determined using a read mapping approach. We build time-resolved phylogenetic trees using BEAST.

**Results**

Our phylogenetic models show that >95% infections within a community are unlinked and that increasing local population size is strongly associated with the number of circulating transmission chains. Nevertheless, there is strong spatial structure: pairs of sequences from the same district had 4.1 times the odds (95% CI: 2.8-6.1) of having a most recent common ancestor within the prior year compared to pairs coming from different ones. This fell to 1 (i.e., no difference) after a period of three years, consistent with it taking three years for Bp to be well-mixed throughout France. Likewise, it takes 5-10 years for Bp to be well-mixed throughout Europe, similar to that observed for the United States. By conditioning on spatial and temporal location of sequences, this approach adjusts for underlying sampling biases.

**Conclusion**

These findings suggest that despite widespread vaccination, there are many independent transmission chains within any location, consistent with widespread asymptomatic carriage. It also suggests Bp strains are able to spread both nationally and internationally in just a few years. These novel insights into Bp dynamics are highly relevant to vaccination policies.
Emergence and dissemination of pertactin-deficient *Bordetella pertussis* carrying an unusual mechanism of pertactin disruption in Spain

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Background

Among the processes of adaptation of *Bordetella pertussis* (*Bp*) to the immunity conferred by acellular pertussis vaccines (ACV), the emergence of strains that do not express the vaccine antigen pertactin (PRN) has been considered. PRN-negative *Bp* isolates have recently increased in countries using ACV, being mostly caused by PRN encoding gene disruption by IS\(^{481}\). The aim of this study is to characterize the PRN-negative *Bp* isolates in Spain, elucidating the temporal evolution of the populations and assessing the impact of the transition from whole-cell vaccine to ACV on their emergence.

Methods

PRN production was studied in 342 *Bp* clinical isolates collected during 1986-2018 from patients diagnosed with pertussis in Spain. The identification of molecular events associated with the loss of PRN production, the genetic relationship between the isolates and the evolution of their population structure were conducted by whole-genome sequencing.

Results

A total of 93 PRN-negative isolates were identified, all detected after ACV introduction, and representing the 38% of the isolates collected during the ACV period. The unusual large deletion *prn*:del(\(-292, 1340)\) on the gene encoding PRN was the most prevalent mutation (58.1% of the PRN-negative isolates). All these isolates belonged to a same genetic cluster, the most recent common ancestor of which is estimated to have occurred in 2007, just after the implementation of ACV in Spain. PRN inactivation by IS\(^{481}\) insertion was identified in 23.7% of PRN-deficient isolates, arising independently multiple times and in different phylogenetic branches.

Conclusions

Our results show how introduction of ACV concurred with emergence of PRN-deficient *B. pertussis* in Spain. Several mechanisms are responsible for this phenomenon; the most identified mutation is *prn*:del(\(-292, 1340)\), found in a specific cluster of *B. pertussis*, which emerged after the implementation of vaccination with ACV. This finding is contrary to what has been observed in other countries, in which an IS\(^{481}\)-mediated PRN gene disruption has been the main mechanism identified. Other factors may have contributed to the dissemination of PRN-deficient isolates in Spain, reinforcing the value of long-term surveillance of *B. pertussis* populations and their antigenic characteristics to assess the role that different pathogen adaptation mechanisms may have in the emergence of pertussis.
Alterations in the expression of *Bordetella pertussis* virulence factors in relation to the use of acellular pertussis vaccine in Finland

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Background
*Bordetella pertussis* isolates which do not express some of acellular pertussis vaccine (ACV) antigens, e.g. pertactin (PRN), have been increasingly reported in countries using ACVs. In Finland, primary pertussis vaccination with whole-cell vaccine was replaced by ACV containing only two components pertussis toxin (PT) and filamentous hemagglutinin (FHA) in 2005. In 2009, the ACV was changed to a three-component vaccine including PT, FHA and PRN. Two years after the change, PRN deficient strains emerged and increased. However, this seems not the case for PT and FHA.

Aim
We aimed to study alterations in the expression of FHA and PRN, two antigens included in ACVs as well as of one important antigen, adenylate cyclase toxin (ACT) not included in current ACVs among Finnish isolates collected during last 30 years.

Methods
Three hundred isolates randomly selected from 950 isolates collected at the Finnish Reference Laboratory for Pertussis and Diphtheria (Turku, Finland) during the period of 1991-2020, were included. An adapted, monoclonal antibody based, antigen expression ELISA, including the culture of *B. pertussis* in Stainer-Scholte medium, was performed to quantify the expression of FHA, PRN and ACT of each isolate. Arbitrary units were used for comparison of each antigen expression by individual isolates.

Results
After the introduction of ACV, the number of PRN deficient isolates has significantly increased. Furthermore, the amount of PRN produced by PRN positive isolates has started to decrease, especially after the use of ACV containing PRN. However, the reverse happened with FHA and ACT. The production of FHA and ACT within the *B. pertussis* strains isolated after 2010 has significantly increased.

Conclusion
The study showed that during the ACV-induced selection pressure *B. pertussis* has changed the production of different antigens. The production of FHA and ACT has highly increased in Finnish *B. pertussis* isolates after the introduction of ACV in this country. This is in line with our previous findings with PT. Our results warrant further studies on production of *B. pertussis* virulence factors in clinical isolates from countries using different ACVs and immunization programmes.
PT makes the nasopharynx to a replication niche for *B. pertussis*, where FhaB and FIM are critical for transmission

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Mouse models of pertussis pneumonia served in characterization of *B. pertussis* virulence mechanisms. However, the biologically most relevant catarrhal disease stage and *B. pertussis* transmission has not been adequately reproduced in adult mice due to limited proliferation of the human-adapted pathogen on murine nasopharyngeal mucosa. We used immunodeficient C57BL/6J MyD88 KO mice to achieve *B. pertussis* proliferation to human-like high counts of $10^8$ viable bacteria per nasal cavity to elicit rhinosinusitis accompanied by robust shedding and transmission of *B. pertussis* bacteria to adult co-housed MyD88 KO mice. Experiments with a comprehensive set of *B. pertussis* mutants revealed that pertussis toxin, adenylate cyclase toxin-hemolysin, the T3SS effector BteA/BopC and several other known virulence factors were dispensable for nasal cavity infection and *B. pertussis* transmission in the immunocompromised MyD88 KO mice. In contrast, mutants lacking the filamentous hemagglutinin (FhaB) or fimbriae (Fim) adhesins infected the nasal cavity poorly, shed at low levels and failed to productively infect co-housed MyD88 KO or C57BL/6J mice. FhaB and fimbriae thus appear to play a critical role in *B. pertussis* transmission.

Moreover, the adenylate cyclase (ACT) and the pertussis (PT) toxins of *Bordetella pertussis* are known to exert potent immunomodulatory activities that synergize to suppress host defense. We thus compared also the mouse lung infection capacities of *B. pertussis* (Bp) mutants (Bp AC- or Bp PT-) producing enzymatically inactive toxoids. Despite accelerated and near complete clearance from the lungs of immunocompetent Balb/c mice by day 14 of infection, the PT- bacteria accumulated within the lymphoid tissue of lung-draining mediastinal lymph nodes (mLNs). In contrast, the wild type or AC- bacteria colonized the lungs but did not enter into mLNs. Lung infection by the PT- mutant triggered an early arrival of migratory conventional dendritic cells with associated bacteria into mLNs, where the PT- bacteria entered the T cell-rich paracortex of mLNs by day 5 and proliferated in clusters within the B-cell zone (cortex) of mLNs by day 14, being eventually phagocytosed by infiltrating neutrophils. Finally, only infection by the PT- bacteria triggered an early production of anti-B. pertussis serum IgG antibodies already within 14 days of infection, indicating that action of the pertussis toxin blocks DC-mediated delivery of *B. pertussis* bacteria into mLNs. Thereby PT action prevents bacterial colonization of mLNs and early adaptive immune response to *B. pertussis* infection, thus enabling high level colonization of the nasopharyngeal mucosa and efficient transmission to new hosts.
Pertussis surveillance and vaccine studies in Sweden

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Background
Sweden discontinued routine childhood vaccination against pertussis between 1979 and 1995. Meanwhile, various efficacy trials were performed in Sweden and elsewhere. The larger of those trials was Stockholm Trial II, which included the whole country of Sweden except for the Gothenburg region. Results for long-term follow-up from Trial II is presented for three different randomised cohorts of children born 1993 and 1994, vaccinated at 3, 5, and 12 months of age. The cohorts received either a five-component acellular pertussis vaccine (aP) (Connaught-Laboratories-Ltd), a three-component aP-vaccine (Chiron-Biocine), or a whole-cell pertussis (wP) vaccine (Evans-Wellcome).

Method
Follow-up- and registry-data were used to investigate vaccine-specific differences in breakthrough cases. Culture-, or PCR-confirmed cases were identified through a mandatory national registry. Interviews were conducted with caregivers. Study records provided vaccine history. Cox-regression was used to pairwise compare the vaccines with the number of months to event defined as the time between the date of the third dose to the date of first day of cough. P-values for proportions were calculated through Chi-squared-tests. Mann–Whitney-U-tests were used to estimate differences regarding time to onset between the vaccines.

Results
Out of 52,818 children who had received three doses of one of the specified vaccines, 312 were diagnosed with pertussis during the 150 months of follow-up. In the wP-group, 0.4% were diagnosed and 0.7% in the aP-groups. The median time from dose 3 to onset was shorter for the 3aP-group and no significant difference was seen between wP- and the 5aP-group. There were significant differences regarding the time to onset for the wP-group compared to the two aP-groups.

Conclusion
In a randomized vaccine efficacy trial long-term surveillance indicated superior protection for one wP-vaccine compared to the two aP vaccines.
**Bordetella pertussis notifications in the Netherlands before and during the COVID-19 pandemic**

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**Background**

Non-pharmaceutical interventions to limit the spread of SARS-CoV-2 have not only resulted in reduced COVID-19 cases, but also lowered transmission of most respiratory-transmitted bacteria and viruses. For some pathogens, like RSV and influenza viruses, this led to disruptions of classical seasonal transmission patterns upon relaxation of COVID-19 control measures. Here, we report on the epidemiology of pertussis before and during the COVID-19 pandemic, and present a novel surveillance approach to monitor the potential rebound of pertussis in the post-pandemic area.

**Materials & method**

Pertussis is a notifiable disease in the Netherlands according to “Wet Publieke Gezondheid”. In consequence, the National Institute of Public Health (RIVM) receives information from all laboratory confirmed cases. In addition, 10,416 combined nasopharyngeal and oropharyngeal swabs, originating from a random sample from the general population between October 2021 and May 2022, were subjected to screening for a large panel of respiratory viruses and bacteria, including *Bordetella*, using the RespiFinder® 2SMART assay. Samples suspected to be *Bordetella* positive were confirmed and typed to species level using a multiplex qPCR targeting IS481, IS1001, and IS1002.

**Results**

The steep decline in the number of notifications that was observed after the introduction of the COVID-19 measures in March 2020 has continued in 2021 and 2022. In 2021, the overall number of pertussis notifications and the incidence rate (IR) were 74 and 0.4 per 100,000 respectively. These unprecedented low numbers compared to the past decades mark a decrease of approximately 99%. In the first seven months of 2022, no increase in the number of pertussis notifications was observed. The additional surveillance of respiratory infections revealed that only 23 out of the 6,128 SARS-CoV-2-negative samples were flagged (potentially) positive for *B. pertussis* of which only one specimen could be confirmed by specific qPCR analysis. These data show the value of multiplex assays for the screening of large samples sets, but indicate importance of confirmation of suspected specimens by specific PCR.

**Conclusion**

COVID-19 related containment measures resulted in a sudden and dramatic drop of pertussis incidence in the Netherlands. Neither the diagnostic laboratory notifications nor the additional surveillance of respiratory infections point towards a resurgence of pertussis in the Netherlands. It is currently impossible to predict when *B. pertussis* will remerge, or which genetic background future strains will have.

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Session 4: Epidemiology
Populational changes of *Bordetella* strains circulating before and during the COVID-19 pandemic period in France

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**Background**

A drastic reduction of outpatient and hospitalized cases of pertussis was observed in France during the COVID-19 pandemic (1). Nevertheless, the French surveillance system of whooping cough also collected non-pertussis *Bordetella* isolates.

**Aim**

We aimed to analyze *Bordetella* isolates collected during COVID-19 pandemic in France from the first lockdown period in March 2020 to May 2022 (26 months) and compare them to isolates collected during the pre-pandemic period from 2018 to February 2020 (27 months).

**Methods**

A total of 243 isolates were collected. All isolates were characterized using classical bacteriological methods. Identification was performed using matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (Bruker). Whole genome sequencing was performed for all isolates (Illumina). Genomic analyses were done using the BIGSdb platform at https://bigsdb.pasteur.fr/bordetella/.

**Results and discussion**

A reduction of the total number of isolates sent to the NRC was observed during the pandemic period: 172 were sent during the 27 months pre-COVID period vs 71 during the 26 months pandemic one. In addition, a change in the main circulating *Bordetella* species was observed. During the pre-COVID period, *B. pertussis* represented 70.8 % of the isolates (120/172) as previously reported in the country (3) whereas it represented only 9.9% during the pandemic period (7/71). In the same way, proportions of *B. holmesii* and *B. trematum* decreased. In contrast, proportions of *B. bronchiseptica*, *B. parapertussis* and *B. hinzii* increased. Phylogenetic analysis based on cgMLST allowed the identification of 2 isolates belonging to a putative novel Genomic Species (GS1) (4); both were collected in the pandemic period and 9 *B. bronchiseptica* isolates out of previously identified *B. bronchiseptica* clusters I and IV (5) collected in both periods. In addition, *B. parapertussis* isolates collected during the pandemic period differed from those that had been collected during the pre-COVID period, most of them being more closed to 12822 reference strain.

**Conclusion**

The number of strains collected by the French NRC for Whooping cough decreased during COVID-19 pandemic. The most frequently collected species differed between both periods, as *Bordetella* species leading to respiratory diseases decreased probably because of measures such as physical distancing.

**References**

Pertussis in Denmark, from high to low: 2019-epidemic and the COVID-19 period

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**Background**
In 2019, Denmark experienced an epidemic of pertussis of a magnitude not seen since the mid-1980s. The epidemic reached the highest peak in January 2020. In March 2020, COVID-19 lockdown was implemented, and the number of confirmed cases of pertussis decreased drastically. The incidence now, in 2022, continues to be very low.

**Methods**
Data on confirmed cases as well as number of diagnostic tests were obtained from the Danish Microbiology Database (MiBa) and linked to other registries. Data for age and geographical location for cases was evaluated, as well as vaccination coverage and hospitalisations for cases among children less than two years of age. The use of diagnostic tests and the corresponding percentage of positive tests was compared to when there was attention to pertussis in the national media.

**Results**
The 2019-20 pertussis epidemic in Denmark reached a peak incidence of 180 per 100,000 per year in the month of January 2020. This was more than tenfold higher than baseline and three times higher than the peak month of the previous epidemic in 2016. After COVID-19 restrictions were imposed, pertussis almost disappeared in Denmark, and the incidence for 2021 was just 1.4 per 100,000 per year. During the epidemic, the proportion of cases among small children was lower, and cases were most likely milder compared to previous periods. The number of diagnostic samples, and therefore the number of cases, increased dramatically in the days after Statens Serum Institut issued newsletters about the high incidence.

**Conclusion**
The 2019-20 pertussis epidemic in Denmark reached very high levels, but a part of this dramatic increase is thought to be due to higher awareness - both because of the fairly recent epidemic in 2016 and also because of high attention in the national media. COVID-19 led to an abrupt end to the epidemic, and the level of pertussis has remained very low since then.
Evaluation of the ESwab® transport system for viability and molecular diagnostics of *Bordetella pertussis*

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Background
Laboratory detection methods for *Bordetella pertussis*, the causative agent of pertussis, include culture and molecular methods. Successful detection requires proper specimen collection and transport. The aim of this study was to evaluate the efficiency of the ESwab® system (Copan Italia, Italy) for transporting and maintaining viability of *B. pertussis* and the suitability of the system for molecular diagnostics.

Methods
The Copan Liquid Amies ESwab transport system (Copan) was validated using the CLSI M40-A2 swab elution method at +4 °C and room temperature (RT) and at three time intervals (0 hours, 24 hours, and 48 hours). A reference strain *Bordetella pertussis* ATCC 9797D was selected for the evaluation. For molecular diagnostics, nucleic acid was isolated from all inoculated ESwab systems and its dilutions using the MagNAPure24 system (Roche, Germany) and real-time PCR was performed using LightMix® Modular *Bordetella pertussis* (Tib MolBiol, Germany).

Results
*B. pertussis* was isolated from all ESwabs after 24 h and 48 h of storage at RT and +4 °C. Bacterial loads (CFU/ml) in samples stored at RT were 9.5×10⁵, 4.1×10⁵ and 9.5×10⁵ at the 0 h, 24 h, and 48 h time intervals, respectively (Table 1). The corresponding loads in samples stored at +4 °C were 8.8×10⁵, 8.7×10⁵ and 9.5×10⁵, respectively (Table 1), and the results were in accordance with CLSI M41-A2 performance standards. Using PCR *B. pertussis* was detected in all ESwab samples, regardless of the initial inoculum and storage conditions.

Conclusion
The ESwab® system is suitable for transport and preservation of viability of *B. pertussis* as well as for use in molecular diagnostics. Transport and storage of the sample can be performed at RT as well as at +4 °C for a period of up to 48 hours.

<table>
<thead>
<tr>
<th>Plate</th>
<th>T=0 h</th>
<th>T=24 h</th>
<th>T=48 h</th>
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<tbody>
<tr>
<td></td>
<td>RT</td>
<td>+4 °C</td>
<td>RT</td>
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<tr>
<td>1,5xE3</td>
<td>1,5*E3</td>
<td>82</td>
<td>1,1E+06</td>
</tr>
<tr>
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<td>1,5*E2</td>
<td>74</td>
<td>7,4E+05</td>
</tr>
<tr>
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<td>1,5*E1</td>
<td>40</td>
<td>4,0E+05</td>
</tr>
<tr>
<td>Average (CFU/ml)</td>
<td>9,5E+05</td>
<td>8,8E+05</td>
<td>4,1E+05</td>
</tr>
</tbody>
</table>

Table 1: Viability of *Bordetella pertussis* after at the 0 h, 24 h, and 48 h storage at room temperature (RT) and +4 °C.
Identification of anti-\textit{Bordetella pertussis} specific antibodies in patients' sera

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Background and aim

Whooping cough is a highly contagious, respiratory infectious disease caused by \textit{Bordetella pertussis}.

The diagnosis methods used are represented by PCR, culture and serology, depending on the cough onset, vaccination status and the age of the patient. The gold standard method used is culture, because of its high specificity, but it can be performed during the first 2 weeks following cough onset. On the other hand, \textit{Bordetella pertussis} grows slowly in culture and the growth is also influenced by the antibiotic treatment of the patient.

Real-Time PCR from nasopharyngeal swab should be the chosen diagnosis method for pertussis in case of a recently vaccinated person, in case of infants aged less than 1 year or if a rapid diagnostic is required. It should be performed within the first month following cough onset, otherwise the result could be false negative.

Serological diagnosis of pertussis infection should be performed at 2-12 weeks following cough onset on serum samples collected from patients who did not recently receive a pertussis vaccine dose or booster.

The aim of this study was to determine IgG and IgA anti-pertussis toxin and anti-hemagglutinin antibody titers, IgG anti-pertactin antibody titers in serum samples and compare the results.

Methods

IgG and IgA anti-pertussis toxin and anti-filamentous hemagglutinin antibody titers and IgG anti-pertactin antibody titers were determined by ELISA. A number of 270 randomly selected serum samples, collected from patients aged 2 months-71 years old were included in the study.

Results

The results showed that 98 (36, 29%) of the tested samples had positive titers for IgG anti-PT (≥100 IU/ml) and 105 (38, 88%) were positive for IgA anti-PT (≥12 IU/ml).

Considering anti-FHA antibodies, 118 (43, 7%) of the tested samples revealed positive IgG titers and 99 (36, 66%) showed positive IgA titers. In case of FHA, results were interpreted as positive or negative, depending on the age of the patients, as recommended in the kit used for specific antibody titer determinations.

In case of PRN, IgG antibodies were determined and the results showed that 8, 51% had titers higher than 100 IU/ml.