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The evolving nature of *Bordetella pertussis* in Ontario, Canada, 2009-2017: strains with shifting genotypes and pertactin-deficiency.

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Abstract

Objective. This study examined the evolving nature of *Bordetella pertussis* in Ontario, Canada by characterizing isolates for their genotypes and expression of pertactin.

Methods. From 2009 to 2017, 413 *B. pertussis* were cultured from pertussis cases at Public Health Ontario Laboratory. Their genotypes were determined by partial gene sequence analysis of their virulence/vaccine antigens: filamentous haemagglutinin, pertactin (PRN), fimbriae 3, and pertussis toxin, including the promoter region. Expression of PRN was measured by Western immunoblot.

Results. Two predominant genotypes, ST-1 and ST-2 were found throughout the study, and were responsible for 47.5% and 46.3% of all case isolates, respectively. The prevalence of ST-1 appeared to fluctuate from 80.3% in 2009 to 20.0% in 2014 and 58.5% in 2017, while the prevalence of ST-2 changed from 18.4% in 2009 to 80.0% in 2014 and 26.2% in 2017. PRN deficient strain was first noted in 2011 (16.7%), and its prevalence increased to 70.8% in 2016 but decreased to 46.2% in 2017. More ST-2 strains (46.6%) than ST-1 (16.8%) were associated with PRN deficiency. Newer ST-21 and ST-22 found in 2015 to 2017 were uniformly PRN deficient.

Conclusion. Impact of the evolving nature of *B. pertussis* on disease epidemiology requires further longitudinal studies.

Introduction

Bordetella pertussis, the causative agent of whooping cough, is a strict human pathogen. In the pre-vaccine era, it mainly affected children under the age of ten, with increased disease activity occurring in a cyclical pattern of every two to five years (Waters and Halperin, 2010). The first generation of pertussis vaccines were inactivated whole cell vaccines, first introduced in the 1940s and that led to dramatic decreases in pertussis disease. More refined and less reactogenic acellular pertussis vaccines (aP) were developed and introduced in Canada in 1997/1998. In the vaccine era, the age distribution of pertussis patients has changed to involve infants (less than one year old), adolescents (10-19 years old) and adults but the cyclic pattern still exist (Guris et al., 1999; Cherry, 2005; Halperin, 2005; Waters and Halperin, 2010).

Pertussis is a reportable disease in Ontario and is controlled by a publicly funded immunization program using the five-component aP (at least for the primary immunization), which consists of pertussis toxin (PT), filamentous haemagglutinin (FHA), pertactin (PRN) and fimbriae 2 and 3 (Fim 2, Fim 3). In Ontario, aP is given at 2, 4 and 6 months of age, with booster doses at 18 months and 4-6 years; and an adolescent dose at 14-16 years of age (Ontario Ministry of Health and Long-Term Care, 2017). Despite vaccination, the cyclical peaks of disease still exist every four to six years in Ontario and between 2007 and 2011, an average of 512 cases were reported every year (Ontario Ministry of Health and Long Term Care, 2014). Beginning in late 2011 and continuing into 2012, a prolonged pertussis outbreak affecting seven health units was reported from southwestern Ontario (Deeks et al., 2014). A smaller regional outbreak has also been reported in the province, e.g. from the North Bay Parry Sound region in 2016 (North Bay Parry Sound District Health Unit, 2017).

78 In the post-vaccine era, a number of studies have shown changes in the *B. pertussis* population
79 (Mooi et al., 1998; Fry et al., 2001; Gzyl et al., 2001). The most dramatic change however, was
80 an increasing number of *B. pertussis* strains not expressing PRN, a component in some
81 formulation of aPs (Otsuka et al., 2012; Lam et al., 2014; Pawloski et al., 2014). The prevalence
82 of PRN-negative isolates and the timing of their appearances appeared to vary widely among the
83 countries reporting such isolates. For example, the first PRN-negative *B. pertussis* was reported
84 from Japan in 1997 (Otsuka et al., 2012), but in Europe, such PRN-negative *B. pertussis* isolates
85 appeared to arise later (in France, 2003; for Sweden and The Netherlands, it was between 2007-
86 2009) (Zeddeman et al., 2014). In Australia their appearance began in 2008 (Lam et al., 2014)
87 and in Canada, the first PRN-negative strain appeared in 2011 (Tsang et al., 2014). In the US, the
88 first PRN-negative pertussis isolate was from 1994 in the state of North Carolina, but no PRN-
89 negative isolates were further found until 2010 when they emerged in larger numbers in multiple
90 states (Pawloski et al., 2014). The prevalence of PRN-negative *B. pertussis* also varied between
91 countries with rates of only 2.6 to 12.3% in France, 4% in Sweden, 25% in Norway (Zeddeman
92 et al., 2014), to 53% in the US (Pawloski et al., 2014) and 78% in Australia (Lam et al., 2014).
93 During the pertussis outbreak in the State of Washington in 2012, 63% of the pertussis isolates
94 collected had been PRN-negative (Bowden et al., 2014). In the State of Vermont where high
95 incidence of pertussis was noted (103 cases per 100,000 population), >90% of their pertussis
96 isolates were PRN-negative (Breakwell et al., 2016). Although the reasons behind this global
97 disparity in the rate of PRN-negative pertussis isolates and the timing of their appearances are
98 not fully understood, it may be worth noting that the kind of pertussis vaccines used by the
99 different countries were probably not the same and might have changed over time even within
100 the same country.

Other changes in the *B. pertussis* population include polymorphisms in its antigen genes such as those included as vaccine components (Mooi et al., 1998; Fry et al., 2001; Gzyl et al., 2001). One such change involves the fimbriae 3 antigen, and the two most common genotypes are *fim3A* and *fim3B*. The difference between these two genotypes is a point mutation that resulted in an amino acid change on a surface epitope which is recognized by human serum antibodies present in subjects infected or vaccinated against pertussis (Tsang et al., 2004; Williamson and Matthews, 1996). Therefore, we hypothesize these polymorphisms may be subjected to immune selection or pressure from antibodies present in the human population, and as such they may fluctuate or alternate between different forms over time to evade immune detection. Newer STs will be expected to emerge as a result.

Previously we studied a convenience sample of *B. pertussis* isolates collected from the period 1994-2013 in Canada, and examined their expression of PRN by Western immunoblot (Tsang et al., 2014). Of the 224 isolates analyzed, only twelve were PRN negative, giving an overall prevalence of 5.4%. However no PRN-negative isolates were detected prior to 2011 and one of ten isolates in 2011 were PRN-negative, which increased to 17.8% (11 of 62 isolates) in 2012. PRN-negative isolates were only found in the provinces of Alberta and Ontario at that time (Tsang et al., 2014).

Recently we carried out a retrospective study that covered the period from 2009 to 2017 in Ontario, Canada to examine the genetic and phenotypic characteristics of all the *B. pertussis* isolates collected at the Public Health Ontario (PHO) Laboratory, Toronto. Our objective was to

examine the emergence and prevalence of PRN-negative pertussis isolates in Ontario, Canada over time. Another objective was to examine if there was any association of PRN-status in isolates and their genotype as determined by partial nucleotide sequence of their virulence/vaccine antigen genes (*prn*, *fhaB*, *fim3*, *ptxS1*, and *ptxP*). We also planned to assess if polymorphisms detected in antigen genes of *B. pertussis* may fluctuate over time, potentially reflecting the response by the bacterium to immune pressure exerted by their human host.

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Materials and Methods

Between January 1, 2009 and December 31, 2017, there were 413 individual *B. pertussis* case isolates available from PHO where culture was performed on PCR positive primary specimens. Isolation of *B. pertussis* at PHO was carried out on charcoal agar plates with 5% horse blood and incubated at 37°C for 72 hours. All cultured isolates were stored frozen (−80°C) at the time of isolation and identification; and vials of frozen cultures were sent to the National Microbiology Laboratory (NML) in Winnipeg, Manitoba in several batches from Spring 2017 to Spring 2018.

At the NML, *B. pertussis* isolates were grown on fresh Bordet-Gengou 15% blood agar plates at 35°C for 48-72 hours and stored at -80°C in Brain Heart Infusion broth + 20% glycerol. Species confirmation was carried out on all isolates and all were confirmed to be *B. pertussis* based on biochemical test results for oxidase, urease, citrate and motility (von Konig et al., 2015).

Serotyping was performed by indirect whole-cell ELISA using commercial antibodies to Fim 2 and Fim 3 (NIBSC, Hertfordshire UK) as previously described (Tsang et al., 2005). Genotyping and sequence type (ST) assignment was determined based on partial gene sequencing for the following virulence/vaccine genes: pertussis toxin subunit 1 (*ptxS1*), pertactin (*prn*), fimbriae 3 (*fim3*), filamentous haemagglutinin (*fhaB*) and the pertussis toxin promoter region (*ptxP*) (Shuel et al., 2013). Detection of PRN was performed at both the NML and PHO by Western immunoblot using commercially available antibodies (NIBSC, Hertfordshire, UK), as previously described (Tsang et al., 2014).

156 Statistical analysis was performed by Fisher exact test (<http://www.socscistatistics.com>) and
157 considered significant if p value was less than 0.05.

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Results

1. Temporal distribution of PRN-negative *B. pertussis* in Ontario.

Of the 413 individual case isolates, 143 (34.6%) were found to lack PRN by Western immunoblot. The distribution of PRN-negative isolates by year is shown in Table 1. In general the percentage of PRN-negative isolates increased yearly since 2011 except in 2017, when its prevalence decreased significantly (p value = 0.01) compared to the data in 2016.

2. Typing of *B. pertussis*

2.1. Serotyping

The majority of the isolates (399 or 96.6%) were serotype 3; only 11 (2.7%) were serotype 2. There was also one isolate (0.2%) typed as serotype 2,3; and two (0.5%) were non-typeable (negative for serotype 2 and 3 antigens). The serotype 2 isolates were found in each year from 2011 to 2017 with one isolate each in years 2011, 2012, 2016 and 2017; two isolates each in years 2013 and 2014; and three in 2015. The non-typeable isolates were found in 2009 and 2012; and the serotype 2,3 isolate was from 2010.

2.2. Genotyping

There were nine sequence types identified among the 413 isolates (Table 2). However, in one isolate there was a 1643 bp deletion involving the promoter region and the 5' end of the *prn* gene identical to the mutation reported previously in an isolate from the U.S. (GenBank accession number CP022362). Since the *prn* deletion in this strain involved the variable region used for genotyping, its *prn* allele and ST could not be determined.

The majority of the isolates (387 or 93.7%) belonged to either ST-1 ($n = 196$ or 47.5%) or ST-2 ($n = 191$ or 46.3%). While ST-1 and ST-2 were found in each year of the study period, their relative frequencies changed over time. Table 3 shows that the peak prevalence of ST-1 (80.3%) was in 2009. ST-1 prevalence then decreased to 20.0% by 2014, and was back to 58.5% in 2017, although numbers were small. Corresponding changes were seen in ST-2; only 18.4% of the isolates in 2009 were typed as ST-2, increasing to 80.0% in 2014, and back to 26.2% in 2017.

During the first five years (2009-2013), six isolates with other STs were found (three ST-8 and one each of ST-11, ST-18 and ST-23). In the next four years (2014-2017), twenty isolates of other STs were found: one isolate of ST-7, nine isolates of ST-21, nine isolates of ST-22; and in the remaining isolate, its ST could not be determined due to a deletion in the region of the gene involved in assigning ST.

Sequence polymorphism was most prominent in the *prn* gene, with seven different *prn* types (Table 4). Both *fhaB* and *fim3* had two allele types each, *ptxS1* had three allele types and *ptxP* had four types (Table 2). The different allele types observed for *ptxS1*, *fhaB*, *fim3* and *ptxP* were based on single point mutations, while variation in the *prn* gene was most often due to deletions or variable number of repeat regions as shown in Table 4.

3. Association of PRN expression and ST

Eighty-nine of 191 (46.6%) ST-2 isolates versus only 33 of 196 (16.8%) ST-1 isolates were PRN-negative (difference was statistically significant, p value <0.001). The number of ST-2 isolates that were PRN-negative appeared to increase steadily each year from 2011 (2/9, 22.2%)

to 2016 (15/15, 100%), and only decreased slightly to 94.1% in 2017 (Figure 1). In contrast the number and percentage of ST-1 being PRN-negative fluctuated between ten isolates or 41.7% in 2016 to only two isolates or 10.0% in 2013. Of the remaining 21 PRN-negative isolates, nine belonged to ST-21, nine belonged to ST-22, one belonged to ST-7, one belonged to ST-11, and in the remaining PRN-negative isolate, it had a *prn* deletion affecting the genotyping region and hence its ST could not be determined. All nine ST-21 isolates were isolated in 2015 to 2017 while the nine ST-22 isolates were recovered in 2016 to 2017.

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Discussion

In this study we confirmed that PRN-negative pertussis strains were first detected in Ontario in 2011, and that their prevalence increased almost yearly from 16.7% (two isolates) in 2011 to 70.8% (34 isolates) in 2016. However, in 2017 we noticed a significant decrease (p value = 0.01) in the prevalence of such isolates to 46.2% (30 isolates). This study also confirmed that over 95% of all the isolates (regardless of PRN status) expressed the Fim3 antigen, which was also observed in isolates recovered between 1998 and 2006 (Shuel et al., 2013). The predominant presence of Fim 3-positive but not Fim 2-positive isolates might be because vaccinated subjects developed a stronger antibody response to the Fim 2 antigen than to the Fim 3 antigen (Hallander et al., 2014), thereby possibly suppressing the transmission of Fim 2-positive isolates.

Besides these phenotypic characteristics, we have also typed the *B. pertussis* by genotyping. We have been collecting genotyping data in Ontario since 1998 (Shuel et al., 2013). During the past 20 years from 1998 to 2017, two STs (ST-1 and ST-2) were found in each of the years we carried out studies (1998-2006; 2009-2017); and they were the predominant STs found throughout the study period. From 1998 to 2006, 94.4% of the 521 isolates analyzed belonged to either ST-1 (*prn2*, *fhaB1*, *fim3B*, *ptxS1A*, *ptxP3*) or ST-2 (*prn2*, *fhaB1*, *fim3A*, *ptxS1A*, *ptxP3*) (Shuel et al., 2013). From 2009 to 2017, 93.7% of 413 isolates were also either ST-1 or ST-2. However, their relative frequencies changed over time. In 1998-2006, ST-1 and ST-2 were responsible for 83.5% and 10.9% of the isolates, respectively. In 2009 to 2017, their relative frequencies were 47.5% for ST-1 and 46.3% for ST-2. The difference between ST-1 and ST-2 is in their *fim3* genes: ST-1 is characterized by the *fim3B* allele while ST-2 is characterized by the *fim3A* allele (Tsang et al., 2004). The *fim3A* allele was detected in the Japanese vaccine strain Tohama and

the reference strain Hav which were isolated in the 1950s or earlier (Tsang et al., 2004). In contrast the *fim3B* allele was first reported in Canada from strains isolated in 1994 (NML unpublished data). It is possible that *fim3B* arose from *fim3A* by a point mutation that effected an amino acid change from alanine (in the Fim3A antigen) to glutamic acid (in the Fim3B antigen) (Tsang et al., 2004). Furthermore, other studies have shown that the affected amino acid maps to a surface epitope recognized by serum antibodies from humans infected with or vaccinated against whooping cough (Williamson and Matthews, 1996). As such, we expect the Fim3A and Fim3B epitopes to be under immune selection. Our laboratory surveillance data on the temporal distribution of ST-1 (*fim3B*) versus ST-2 (*fim3A*) appears to support this hypothesis when ST-1 disappears to give way to ST-2 and vice versa (Table 3).

In addition to ST-1 and ST-2, nine other STs (29 isolates) were identified during 1998-2006 (Shuel et al., 2013). These STs include ST-5, ST-6, ST-7, ST-8, ST-9, ST-10, ST-11, ST-12 and ST-16. However, in the subsequent period (2009-2017), only ST-7 (one isolate), ST-8 (three isolates) and ST-11 (one isolate) were still present, but in small numbers. This suggests that these non-ST-1/ST-2 strains were either circulating in very low numbers (in some cases non-detectable) or are less fit for survival / transmission. In the period 2009 to 2017, there were seven STs (25 isolates) other than ST-1 and ST-2 including ST-8 (three isolates), ST-21 (nine isolates), ST-22 (also nine isolates), and one isolate each of ST-7, ST-11, ST-18, and ST-23. Both ST-21 and ST-22 appeared to be newer STs since they were isolated only between 2015 and 2017; and may be increasing (3.2% in 2015, 14.6% in 2016, and 15.3% in 2017). Continued surveillance is important to determine if these newer ST-21 and ST-22 will continue to expand and contribute as the primary STs causing pertussis.

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261 Another important finding of this study was the apparent association of ST-2 with the PRN-
262 negative status (89/191 isolates or 46.6%) versus ST-1 (33/196 isolates or 16.8%). Also the
263 newer ST -21 and ST-22 which were only found in 2015-2017 were uniformly PRN-negative.
264 Since the association of ST-2 with PRN-negative status is not absolute, another contributing
265 factor may well be temporal since over half of 2015 and 2016 isolates were PRN-negative.
266 Nevertheless, there may be an apparent co-existence of fewer PRN-negative isolates and fewer
267 ST-2 strains in Ontario in 2017. A recent significant decrease in PRN-deficient pertussis isolates
268 has also been observed in Japan (Hiramatsu et al., 2017). The authors attributed this decrease to a
269 change in the vaccine formulation that did not include PRN which might alleviate the vaccine
270 pressure on the pertussis population. The authors also noted a change in the pertussis genotype to
271 one that was rarely associated with PRN-deficiency. Since the decrease in prevalence of PRN-
272 deficient strains began in 2008-2010 with a further decrease observed in 2011-2013 and the
273 vaccine change was introduced in November 2012, the significant change in PRN deficiency rate
274 was more likely due to a change in the strain genotype that did not carry PRN-deficiency. A
275 change in the pertussis strain genotype and a significant drop in the PRN-negative rate were also
276 observed in Ontario in 2017 when compared with the data from 2016 (p value for the differences
277 in PRN-negative rates = 0.01). Therefore, it is important to continue our laboratory surveillance
278 to see if the downward trend in PRN-negative isolates continues as well as the changeover in
279 their genotype.

280

281 Our current data as well as previous results (Shuel et al., 2013; Tsang et al., 2014) suggested that
282 the *prn*, *fhaB*, *fim3*, *ptxS1* and *ptxP* mutations that occurred in strains other than ST-1 or ST-2

were either not stable or did not impart a survival advantage that would allow them to transmit and cause a noticeable amount of disease as evidenced by the relatively smaller number of cases they caused. In contrast, strains with *fim3A/fim3B*, *ptxS1A*, *ptxP3* or PRN-deficiency mutations appeared to be stable and they (as exemplified by ST-1 and ST-2 as well as ST-21 and ST-22 from 2015 to 2017) caused the bulk of pertussis disease in this study and our earlier studies (Shuel et al., 2013; Tsang et al., 2014). PRN deficiency has been suggested to be the result of vaccine pressure and the PRN-negative strains have been described to have a selective advantage especially in the presence of aP pressure (Hegerle et al., 2014; Safarchi et al., 2015; Martin et al., 2015). However, aPs have been said to induce protective immunity of limited duration (McGirr and Fisman, 2015; Warfel and Edwards, 2015), and some components like Fim 2 may induce a stronger immune response than Fim 3 (Hallander et al., 2014). Natural immunity induced by circulating pertussis strains is also likely to play a role in exerting immune selection on the pertussis bacteria. Both vaccine pressure and natural immunity in the population may be responsible for the emergence and possibly subsequent decrease in the prevalence of PRN-negative strains as well as the alternating dominance of ST-1 (*fim3B*) and ST-2 (*fim3A*) strains observed in Ontario.

One limitation of our study was the small number of positive cultures available in some years. Furthermore, we did not have data on outbreak information to link outbreaks with strain types, nor have we examined the types of *prn* mutations that were associated with PRN-deficiency. Nevertheless the current study provides a good foundation for us to carry out our second stage of analysis which will examine the demographics including vaccine eligibility of different birth-cohorts, geographical distribution of PRN-deficient isolates, the kind of mutations in the *prn*

genes contributing to or responsible for PRN deficiency, and more in-depth genetic characterization from whole genome sequence analysis. Eventually it will be essential to evaluate the impact of these changes, if any, on vaccine effectiveness.

In conclusion, we have demonstrated a yearly increase in PRN-negative *B. pertussis* strains in Ontario over the past six years (from 2011 to 2016), and a shifting genotype involving ST-1 and ST-2, which predominated over the study period. Newer STs were found in recent years (2015-2017) which were associated with PRN-deficiency but their stability and ability to become major causes of pertussis remain unknown and warrant further studies. Therefore, a public health surveillance system on pertussis should include a strong laboratory component that includes collection and characterization of representative isolates so that epidemiological data and vaccine effectiveness can be studied according to strain characteristics.

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Conflict of interest. The authors have no conflict of interest to declare.

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Figure Legend

Figure 1. Number of PRN-positive and PRN-negative *Bordetella pertussis* isolates by sequence type (ST) and year of isolation.

Table 1. Number and percentage of pertactin (PRN)-positive and PRN-negative* *Bordetella pertussis* isolates each year in Ontario, Canada from 2009 to 2017.

Year	Number of PRN positive Isolates (%)	Number of PRN negative Isolates (%)	Total Number of Isolates
2009	76 (100)	0 (0)	76
2010	16 (100)	0 (0)	16
2011	10 (83.3)	2 (16.7)	12
2012	45 (64.3)	25 (35.7)	70
2013	44 (67.7)	21 (32.3)	65
2014	18 (60.0)	12 (40.0)	30
2015	12 (38.7)	19 (61.3)	31
2016	14 (29.2)	34 (70.8)	48
2017	35 (53.8)	30 (46.2)	65
Total	270 (65.4)	143 (34.6)	413

* determined by Western immune-blot using commercial antiserum to pertactin

Table 2. Association of multi-locus sequence type (ST) and pertactin (PRN) status by Western blot among 413 *Bordetella pertussis* isolates in Ontario, Canada.

ST	Total Number of Isolates	Number of PRN-positive	Number of PRN-negative	<i>ptxS1</i> Allele	<i>prn</i> Allele	<i>fhaB</i> Allele	<i>fim3</i> Allele	<i>ptxP</i> Allele
1	196	163	33	<i>ptxS1A</i>	<i>prn2</i>	<i>fhaB1</i>	<i>fim3B</i>	<i>ptxP3</i>
2	191	102	89	<i>ptxS1A</i>	<i>prn2</i>	<i>fhaB1</i>	<i>fim3A</i>	<i>ptxP3</i>
7	1	0	1	<i>ptxS1A</i>	<i>prn3</i>	<i>fhaB1</i>	<i>fim3B</i>	<i>ptxP3</i>
8	3	3	0	<i>ptxS1A</i>	<i>prn9</i>	<i>fhaB1</i>	<i>fim3B</i>	<i>ptxP3</i>
11	1	0	1	<i>ptxS1B</i>	<i>prn1</i>	<i>fhaB1</i>	<i>fim3A</i>	<i>ptxP1</i>
18	1	1	0	<i>ptxS1D</i>	<i>prn7</i>	<i>fhaB2</i>	<i>fim3A</i>	<i>ptxP2</i>
21	9	0	9	<i>ptxS1A</i>	<i>prn19</i>	<i>fhaB1</i>	<i>fim3B</i>	<i>ptxP3</i>
22	9	0	9	<i>ptxS1A</i>	<i>prn4</i>	<i>fhaB1</i>	<i>fim3A</i>	<i>ptxP3</i>
23	1	1	0	<i>ptxS1A</i>	<i>prn2</i>	<i>fhaB1</i>	<i>fim3B</i>	<i>ptxP21</i>
ND*	1	0	1	<i>ptxS1A</i>	ND*	<i>fhaB1</i>	<i>fim3A</i>	<i>ptxP3</i>

* Not determined due to deletion of the *prn* variable region

Table 3. Temporal distribution of multi-locus sequence types (ST) of *Bordetella pertussis* in Ontario, Canada.

Year of Isolation	Number (%) of Isolates by Multi-Locus Sequence Type (ST)			Total Number of Isolates
	ST-1	ST-2	Others*	
2009	61 (80.3)	14 (18.4)	1 (1.3)	76
2010	3 (18.8)	9 (56.3)	4 (25.0)	16
2011	3 (25.0)	9 (75.0)	0 (0)	12
2012	29 (41.4)	41 (58.6)	0 (0)	70
2013	20 (30.8)	44 (67.7)	1 (1.5)	65
2014	6 (20.0)	24 (80.0)	0 (0)	30
2015	12 (38.7)	18 (58.1)	1 (3.2)	31
2016	24 (50.0)	15 (31.3)	9 (18.8)	48
2017	38 (58.5)	17 (26.2)	10 (15.4)	65
Total	196 (47.5)	191 (46.3)	26 (6.3)	413

* Other STs included ST-8 (n=3, 2009-2010), ST-18 (n=1, 2010), ST-23 (n=1, 2010), ST-11 (n=1, 2013), ST-21 (n=9, 2015-2017), ST-22 (n=9, 2016-2017), ST-7 (n=1, 2016) and one that could not be typed due to deletion of the *prn* variable region (2016).

Table 4. Differences in the 7 different pertactin (*prn*) gene alleles found in *Bordetella pertussis* isolates in Ontario, Canada.

<i>prn</i> Allele	Genbank Accession Numbers	Position and Sequence		
		666	826	856
<i>prn1</i>	AJ011091	GTG TTG GGG GCC AGT V L G A S	GGT GGT GCG GTT G G A V	GGC GGC TTC GGT CCC G G F G P
<i>prn2</i>	AJ011092	GTG TTG GGG GCC AGT V L G A S	GGC GGC TTC GGT G G F G	GGC GGC TTC GGT CCC GGC GGC TTC GGT CCC G G F G P G G F G P
<i>prn3</i>	AJ011093	GTG TTG GGG GCC AGT V L G A S	GGC GGC TTC GGT G G F G	GGC GGC TTC GGT CCC G G F G P
<i>prn4</i>	AJ011015	GTG TTG GGG GCC AGT V L G A S	GGC GGC TTC GGT G G F G	
<i>prn7</i>	AJ133784	GTG TTG GGG GCC AGT V L G A S	GGT GGT GCG GTT G G A V	GGC GGC TTC GGT CCC G G F G P
<i>prn9</i>	AJ315611	GTG TTG GGG GCC AGT V L G A S	GGC GGC TTC GGT G G F G	GGC GGC TTC GGT CCC GGC GGC TTC GGT CCC GGC GGC TTC GGT CCC G G F G P G G F G P G G F G P
<i>prn19*</i>	Submitted 2017-10-10	GTT GGG GGC CAG TGA V G G Q .		

* GT deletion at position 666, resulting in premature stop codon (.) at amino acid 226

Figure 1. Number of PRN-positive and PRN-negative *Bordetella pertussis* isolates by sequence type (ST) and year of isolation.

* ST not determined for one isolate due to deletion of the *prn* variable region