

# Porcine Reproductive and Respiratory Syndrome virus (PRRSV) regional vaccination program in the Beauce area in Quebec

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Summary

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## 1 Project goal

The main goal of the project was to evaluate the efficiency of a regional vaccination approach using an autogenous killed virus vaccine to control the Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) with the hopes of developing new prevention tools for the illness.

## 2 Methodology

The study was carried out over the course of two years, from September 2008 through September 2010. We selected a total of 40 herds. The base unit for the experiment was the sow herd. The herds were located in the Beauce region of Quebec, more specifically in the villages of Saint-Bernard, Saint-Elzéar-de-Beauce, Saint-Narcisse-de-Beaurivage and Saint-Patrice-de-Beaurivage. We identified twenty control herds and twenty herds where the sows received the autogenous vaccine. The decisions concerning which farms were “control” and which were “vaccinated” was made according to convenience. Biosecurity measures in place on control and “vaccinated” farms were compared using Production Animal Disease Risk Assessment Program (PADRAP) tool.

A sub-group of five “sentinel” farms was randomly chosen for each treatment group. On each of these sentinel farms, a group of 40 sows was selected to be tracked over the whole course of the project in order to study and improve the understanding of the immune response of the animals to PRRSV.

For each treatment group, all sows had to be exposed to the PRRSV virus before the beginning of the project. The exposure to PRRSV was either as a result of an injection of the commercial vaccine Ingelvac PRRS MLV or Ingelvac PRRS ATP or the result of an outbreak of PRRS. The sows from the “vaccinated” group of farms were injected with the experimental autogenous vaccine every six months whereas the sows from the control farms were not vaccinated against PRRS with the autogenous vaccine (Table 1).

Table 1 Vaccination schedule

Event	Day	Vaccine	Approx. date	Comments
First vaccination	0	1 <sup>st</sup> batch	September 2008	
Booster	30	1 <sup>st</sup> batch	Oct. to Nov. 2008	2 to 5 weeks after the 1 <sup>st</sup> vaccination
Second vaccination	180	2 <sup>nd</sup> batch	March 2009	
Third vaccination	360	3 <sup>rd</sup> batch	September 2009	
Fourth vaccination	540	4 <sup>th</sup> batch	March 2010	
			September 2010	End of project

During the study, a record of the most important health events occurring at the herd level was registered. Information came from the producers and from the attending veterinarians. When an outbreak of PRSS was suspected, two diseased piglets were sent to the provincial pathology lab (Laboratoire d'expertise en pathologie animale du Québec - LEPAQ), organs and tissue were analysed for the presence of macroscopic and microscopic lesions compatible with PRRS virus infection and the presence PRRS virus in the tissue was checked using Polymerase Chain Reaction (PCR). When the results confirmed PRRS virus infection (microscopic lesions and identification of PRRS virus), the 603 bases of the Open Reading Frame 5 (ORF5) of the virus were sequenced. Based on the ORF5 sequence, homology tables and dendrograms were constructed to compare PRRS viruses. Two viruses were considered to be identical when the homology was higher than 98%, similar when homology was between 92 and 98%, and,

different below 92%. Moreover, at the end of the study, the PRRS viruses were reclassified according to RFLP (Restriction fragment length polymorphism) patterns using the ORF5 sequences.

On the sentinel farms where animals had been vaccinated, blood tests were carried out on the sows before and around 30 days after each vaccination that occurred every six months (9 serums per sow). On the control sentinel farms, blood tests were carried out every six months (5 serums per sow). Serums from 15 sows with complete samplings in the control (5 per sow) and “vaccinated” groups (9 per sow) were submitted to a laboratory in order to check for the presence of anti-PRRS antibodies and in order to quantify (semi-quantitative) them. Two technologies were used: the presence of non-specific anti-PRRS antibodies was tested using the ELISA technique (IDEXX PRRS-2XR) and the presence of antibodies against four strains of viruses included in the autogenous vaccine was tested by sero-neutralisation (Newport Laboratories technology).

Two methodologies were used to evaluate the stability of production at the farm. These methods allowed us to identify periods where production was considered stable and periods where production was lower than expected or the farm was exposed to a disease outbreak. These methods were: 1) Producer’s Perception of an outbreak along with Laboratory analyses (PPL methodology) and 2) Analysis of the production Data (DA methodology) from the producer livestock-production-software.

According to the PPL methodology, a farm was considered to be going through a PRRS outbreak when the producer noticed clinical signs compatible with PRRS infection and when the presence of the disease was confirmed by laboratory analyses (tissue lesions compatible with the disease and the presence of the virus). The beginning and the ending of the period of disease outbreak were defined by the producer.

For each farm, production data were handled to show temporal variation of weaned piglets per bred sow (novel indicator), weaned/born alive (survival rate), born alive/total born, total born/litter, litter/breeding (see example at Figure 1). Herd performances 18 months before (January 2007 – August 2008) and after the project started (December 2008 – July 2010) were compared in order to test the efficiency of the autogenous vaccine. A period of three of 3 months (September 2008 – November 2008) was excluded from the analysis. This period was considered to be a buffer period to allow vaccination to take effect.

The number of “piglets weaned per bred sow” (more specifically, per service) is a novel indicator developed specifically to monitor losses related to PRRS in sow units. Indeed, this indicator integrates lower herd performances related to all recognized clinical signs associated with PRRS circulation (sow abortion, premature farrowing, sow death, sow fertility problems, mummified piglets, born dead piglets and pre-weaning mortality).

Based on the indicator “piglets weaned per service”, a statistical methodology was used to identify periods of lower production (red dots on Figure 1). A period of lower production, using DA methodology, was defined as a period of at least two consecutive temporal data points with lower production. The detection of periods of lower production also allowed the calculation of unproduced piglets during these periods. In the prospective part of this project (September 2008 - August 2010), most of these low production periods (DA methodology) could be related to PRRS outbreaks (PPL methodology). Therefore, the unproduced piglets during these periods of low production (DA methodology) were integrated in the economic models to estimate the cost of PRRS before (January 2007 - August 2008) and after vaccination (September 2008 - August 2010).



The two methodologies used to define problem periods (low production with DA methodology and PRRS outbreaks using PPL methodology) allowed identifying periods of normal and low productions (DA) and periods of PRRS asymptomatic and PRRS outbreak. Overlapping results from both methodologies allowed visual assessment of temporal agreement of both methods. Agreement between both estimates of production problems has also been tested using Cohen's Kappa statistic.

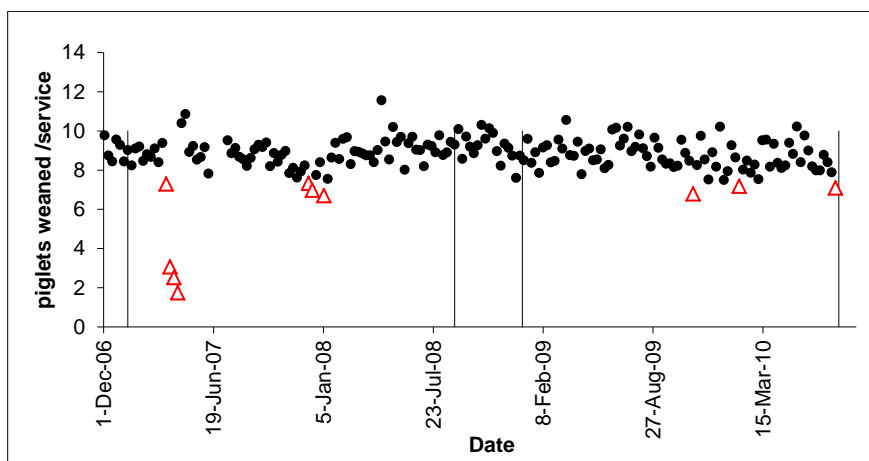


Figure 1 Overview of production losses on a farm before and after vaccinations

### 3 Results and discussion

#### 3.1 Overall description

##### 3.1.1 Participating farms

At the start of the project (August 2008), 40 farms were selected. However, within three months, four farms went out of business. Participating farms (n=36) were either maternities (M) or farrow to finish (F-F) operations (Table 2). Each type of farms was equally distributed among both treatments. Moreover, at the end of the project (last quarter), two other farms stopped production. Finally, production data of one participating farm had to be excluded from performance analysis.

This project was an innovation for the participating producers (>36) and their veterinarians (>9). Indeed, participating producers accepted to share information about the circulation of PRRS virus between the participating farms, they accepted to share the PRRS viruses to allow their inclusion in the common autogenous vaccine, and, finally, they accepted to share their production data to estimate the efficiency of the vaccination. Retention rate of participating farmers in this project was very high (90% - 36/40 farms).

Table 2 Participating farms during this project (Maternity only – M; Farrow to Finish – F-F)

Project stage	Controls			Vaccinated			All		
	M	F-F	TOT.	M	F-F	TOT.	M	F-F	TOT.
Sept. 2008, Startup	5	15	20	5	15	20	10	30	40
Dec. 2008, Adjustments	5	13	18	5	13	18	10	26	36
Sept. 2009, Midway	5	13	18	5	13	18	10	26	36
August 2010, End	5	12	17	5	12	17 <sup>1</sup>	10	24	34
Production data analysis	5	12	17	5	11	16	10	23	33

1 – Production data from one of these farms could not be analysed.

### 3.1.2 Geolocation

Participating farms were located in the Beauce region of Quebec, more specifically in the villages of Saint-Bernard, Saint-Elzéar-de-Beauce, Saint-Narcisse-de-Beaurivage and Saint-Patrice-de-Beaurivage. The 36 participating farms were all located in an area of approximately 20 km in diameter (area of 314 km<sup>2</sup>; Figure 2). This area is very densely populated with pig farms (approximately 1.4 pig sites per km<sup>2</sup>). Geographical data analysis of all pig farms in the zone is suggesting that there are more than 400 units in the zone.

Consequently, the 36 participating farms, although located in a small area (20 km in diameter) represented only a small proportion of the farms in the zone (36/400 = 9%).



Figure 2 Geolocation of the 36 participating herds (18 control herds “T” and 18 vaccinated herds “V”)

### 3.1.3 Herd sizes

Herd sizes, based on the number of sows in inventory, varied between 100 and 2 000 sows (Table 3). Sow herd sizes and total number of sows were similar between both groups ( $P > 0.5$  Khi-square test).

Table 3 Distribution of the herd size and the total number of sows in both treatments

Startup (2008)	Controls		Vaccinated		Total	
Herd size	N	Sows	N	Sows	N	Sows
100-299	10	1 848	9	1 805	19	3 653
300-799	8	3 398	7	2 820	15	6 218
800-2000	2	2 490	4	5 820	6	8 310
Total	20	7 736	20	10 445	40	18 181

2009-2010	Controls		Vaccinated		Total	
Herd size	N	Sows	N	Sows	N	Sows
100-299	9	1 540	9	1 835	18	3 349
300-799	7	3 015	5	2 230	12	5 245
800-2000	2	2 540	4	5 801	6	8 341
Total	18	7 095	18	9 866	36	16 935

### 3.1.4 Preparation of the autogenous vaccines

Autogenous vaccines are prepared from the culture of the viruses circulating in the farms or the production systems. Autogenous vaccines are prepared from killed virus. It is technically possible to include more than one strain in the same vaccine (two to eight strains). In Canada, preparation of autogenous vaccines is regulated by the Canadian Food Inspection Agency (CFIA). Normally, autogenous vaccines are restricted to the production system that provided the virus.

The preparation and the use of an autogenous vaccine from 40 production sites required a special licence. The delivery of a licence by CFIA for this project was a Canadian innovation. All the participating farms were considered as stakeholders of the same production system.

When this project started, there was not any certified laboratory in Canada that had the expertise and the required licences to prepare an autogenous killed virus vaccine. The vaccines for this project were prepared by a certified laboratory in the USA (Newport Laboratories).

When the project began, the scientific team of the project planned for four batches of autogenous vaccines (two per year) with different viruses. Technically, the vaccine was supposed to be renewed with new strains every six months. For unexplained reasons, only a few (8/73 virus  $\cong$  10%) isolated viruses from the zone could be efficiently grown on cell lines. This technical problem greatly limited the choice of strains for vaccine production.

The first two vaccines contained the same strains. Homology table shows that most (3 out of 4) strains included in the vaccines were very different (<92% homology - Table 4).

Table 4 Homology of virus strains contained in the three autogenous vaccines

Autogenous vaccine in batch n° 1 (Sept. 2008) and n° 2 (April 2009)				
	S7-1043	S7-1215	S7-1044	S7-1201
S7-1043	1	0.84	0.86	0.86
S7-1215		1	0.86	0.88
S7-1044			1	0.94
S7-1201				1
Autogenous vaccine: batch n° 3 (Sept. 2009)				
	S7-1044	S7-1201	S8-0789	1131949
S7-1044	1	0.94	0.87	0.87
S7-1201		1	0.89	0.91
S8-0789			1	0.89
1131949				1
Autogenous vaccine: batch n° 4 (April 2010)				
	S8-0789	1131949	S8-0840	1155278
S8-0789	1	0.88	0.87	0.88
1131949		1	0.85	0.85
S8-0840			1	0.82
1155278				1

It is difficult to understand the low success rate of virus cultivation in this project. Indeed, based on experience in other production systems (Newport Laboratories expertise), expected success rate is usually over 50%. It is possible that the Quebec strains are different or that the sample preparation process reduced virus viability. In fact, in order to allow this project to be carried out, all the tissue samples were frozen in Canada and send in batches to the United States. It was impossible to work with fresh tissue because of the distance between the collection zone (Quebec - Canada) and the lab (Minnesota - USA).

### 3.1.5 Biosecurity level

Biosecurity measures in place on control and “vaccinated” farms were compared using the PADRAP tool. The biosecurity level, as estimated by the PADRAP tool, was similar among “vaccinated” and control farms (Table 5). This indicates that the treatment groups should be comparable.

Table 5 Risk index of major factors that may promote spread of the PRRS virus on farms

Risk factors	Risk index (average)		
	All (n = 36)	Control (n = 18)	Vaccinated (n = 18)
Nearby health threats	55.46	54.05	56.88
Nearby hog sites and roads	43.58	44.00	43.15
Replacement animals	35.55	34.38	36.72
Manure management	30.04	29.19	30.89
Other vectors	25.42	25.30	25.54
Dead stock	24.01	23.87	24.16
Transportation	23.30	23.50	23.09
Site and housing	20.94	21.35	20.54
Herd demographics	19.33	18.43	20.23
PRRS status	17.10	13.33	20.87
Semen	15.84	14.81	16.88
Viral exposure strategies	13.15	15.23	11.08
Management of human resources	9.70	9.91	9.48

Investigation of biosecurity practices using PADRAP showed that major biosecurity problems in the Beauce region are linked to geographical density of pig sites and to the management of replacement animals, manure disposal, dead animals and animal transportation (Table 5).

### 3.2 PRRS related outbreaks

Surveillance strategy on the participating farms allowed the construction of a dashboard showing temporal variation of PRRSV herd health status. As described earlier, all the sow herds were contaminated with wild strains of PRRS. Surveillance allowed showing the duration of the outbreaks in the participating herds (Figures 3-4).



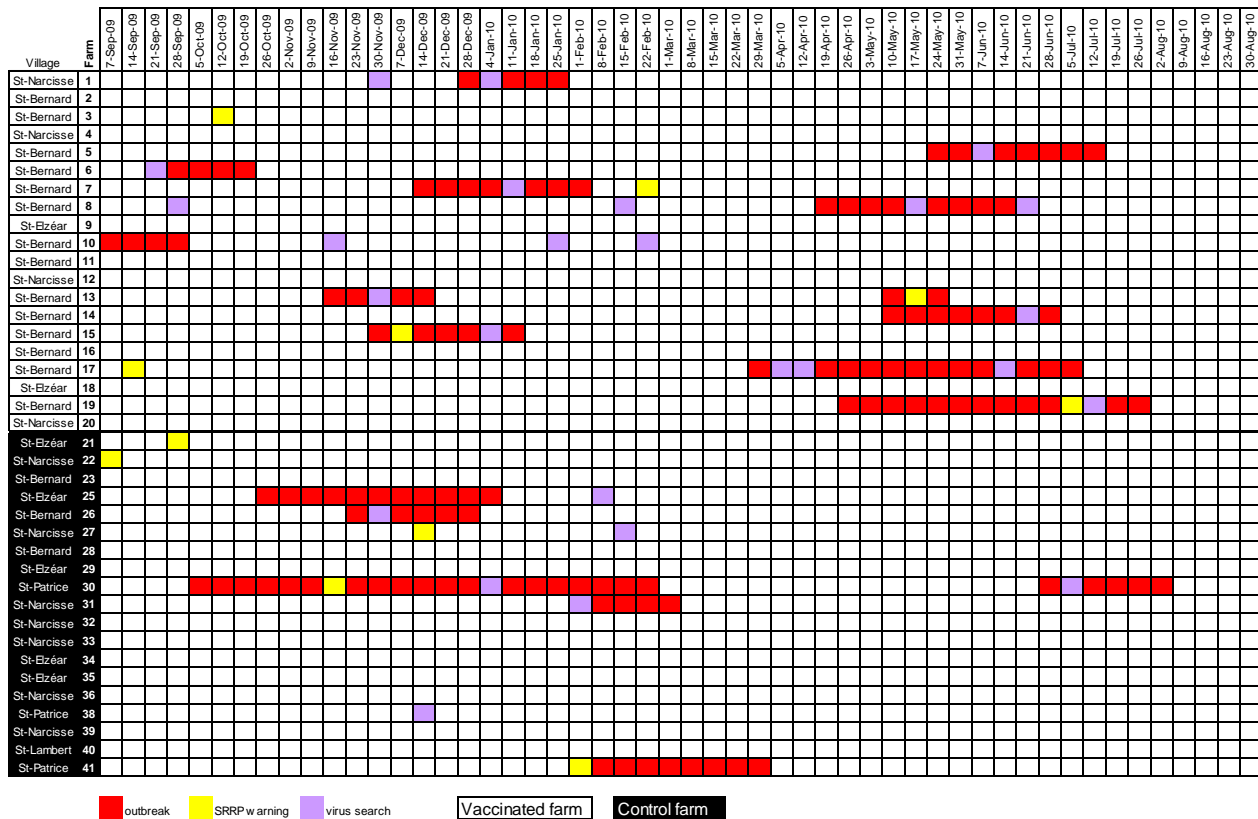


Figure 4 PRRS virus outbreaks among the participating herds, as perceived by the producers, during the second year of the project (2009-2010, 36 sites)

Odds of PRRS outbreaks were high among participating farms (annual rate between 33 and 72% - Table 6). During the prospective part of the project (2008-2010), there were fewer PRRS outbreaks on the control maternity sites (15 outbreaks) than on the sites where the animals had been vaccinated (23 outbreaks).

Table 6 Description of the number of outbreaks (PPL methodology) among participating farms monitored during the two years

Parameter	2008-2009		2009-2010		2008-2010	
	Vaccinated	Controls	Vaccinated	Controls	Vaccinated	Controls
Outbreaks	13	9	10	6	23	15
Farms	18	18	18	18	36	36
Proportion	72%	50%	56%	33%	64%	42%

PRRS outbreaks, although common among the 36 participating farms, were not uniformly distributed. Over the 2 years of the project, half went through a PRRS outbreak while nine had no outbreak at all (25%). A quarter of the sites experienced two or three outbreaks during the monitoring period (2008-2010). Vaccination with the autogenous PRRS killed virus vaccine did not significantly reduce the odds of PRRS outbreaks among “vaccinated” farms.

### 3.3 PRRS virus circulation in the zone

PRRS virus detection (PCR) and sequencing in the zone were quite successful. From September 2008 to August 2010, there were 94 submissions for PRRS virus identification (PCR). Seventy three cases were found positive (73/94, 78%). From these PRRS positive cases, 55 contained a sufficient quantity of virus to be submitted for sequencing (603 bases of ORF5). Success rate of the sequencing procedure was high: 91% (50 sequences/55 trials).

Although success rate for PRRS detection (PCR) and sequencing were high, success of viral culture on cell lines to prepare the autogenous vaccines was low (~ 10%, see “Preparation of the autogenous vaccine”).

Based on the ORF5 sequence, homology tables and dendrograms were constructed to compare PRRS viruses. In spite of the large number of virus identified in the zone (>50), only six strains of identical viruses (>98% homology, according to the classification of the 603 virus bases) were found on more than one site and only one strain was identified on more than two sites. Classifying the strains with the RFLP methodology suggests a larger circulation of similar strains of the virus (8) in the zone. Finally, the viruses included in the autogenous vaccines have never been found a second time in the herds from the zone.

The very large number of different strains of PRRS virus isolated in such a small territory (20 km in diameter) suggests a great diversity of strains in the Beauce region. Moreover, this great diversity of virus suggests there might be more than one strain circulating on the same farm. In fact, the probability of finding the PRRS virus from piglet tissue was the same whether the farm was experiencing a PRRS outbreak or whether it was deemed to be stable (around 80%). Finally, we can conclude that the number of PRRS viruses in circulation in the Beauce area is probably far higher than what is suggested by the number of outbreaks.

### 3.4 Immune response to vaccination

Serums from 15 sows with complete samplings in the control (5 per sow) and vaccinated group (9 per sow) were submitted to a laboratory in order to check for the presence of anti-PRRS antibodies and quantify them (semi-quantitative). Two technologies were used: the presence of non-specific anti-PRRS antibodies was tested using the ELISA technique and the presence of antibodies against four strains of viruses included in the autogenous vaccine was tested by sero-neutralisation.

Results show that sows' serum titers for PRRS viruses increase following vaccination. This is suggesting that the autogenous vaccine does indeed stimulate the sows' immune systems. It is possible that the herds where the animals had been vaccinated were protected against the virus contained in the vaccine (homologous protection), but that the stimulation that resulted from introduction of the autogenous virus was not enough to provide overall protection (heterologous protection) against the large number of viruses circulating in the zone.

### 3.5 Autogenous vaccine for Area regional control of PRRS

The large number and diversity of PRRS virus strains detected in the zone (more than 45 different strains) were far beyond the conceptual framework of an autogenous vaccine (two to eight strains per vaccine). Assuming there was no technical problems with PRRS virus culture, more than 20 strains of virus should have been included into the vaccines to ensure homologous protection for the majority of the viruses circulating in the zone.

In spite of the results from this project, regional-scale vaccination using autogenous vaccines remains an option that must be considered in certain situations. Indeed, beneficial results might be expected in some areas where there are only a few strains of PRRS virus circulating between farms.



### 3.6 Outbreaks and periods of low production

The two methodologies used to define problem periods (low production with DA methodology and PRRS outbreaks using PPL methodology) allowed identifying periods of normal and low productions (DA) and periods of PRRS asymptomatic and PRRS outbreak. Overlapping results from both methodologies allowed visual assessment of the agreement of outbreaks and periods of lower production.

Agreement between PRRS outbreaks, as perceived by the producer and confirmed by laboratory procedures, and period of low production, as detected by production data analysis, is good (Cohen's kappa = 0.58 with a proportion of agreements of 80% - Table 7).

The novel indicator, weaned piglets per service (WPPS), was found to be a very sensible indicator to detect production problems related to PRRS outbreak (Figure 1). Indeed, this indicator integrates lower herd performances related to all recognized clinical signs associated with PRRS circulation (sow abortion, premature farrowing, sow death, sow fertility problems, mummified piglets, born dead piglets and preweaning mortality). The indicator 'weaned piglets per service' was used to estimate periods of low productivity with the data analysis methodology.

Most of the periods of low production (20/25 = 80%), detected by time series analysis of the WPPS indicator calculated from the data obtained from the producer livestock production software had been reported by the producer as PRRS outbreaks. Five drops in production detected by the DA method were not reported by the producer as being related to PRRS. Finally, several of the PRRS outbreaks perceived by the producer and confirmed by detection of the presence of the virus (PPL method) were not detected by the DA method (11/31 = 35%).

Table 7 Agreements between PRRS outbreaks, as Perceived by the Producer and confirmed by Laboratory procedure (PPL procedure) and periods of low production identified by production Data Analysis (DA procedure)

Outbreaks (PPL procedure)	Lower production (DA procedure)		Total
	Yes	No	
Yes	20	11	31
No	5	50	55
<b>Total period</b>	<b>25</b>	<b>61</b>	<b>86</b>

The good correlation between drops of production and PRRS virus outbreaks is suggesting that the number of unproduced piglets, estimated from the period of low production, can be used as an estimate of the impact of PRRS outbreaks on the productivity of the herds.

### 3.7 Productivity impact of PRRS virus outbreaks

Herd technical performances, 18 months before (January 2007 – August 2008) and after the project started (December 2008 – July 2010), were compared in order to test the efficiency of the autogenous vaccine. A period of three months (September 2008 – November 2008) was excluded from the analysis. This period was considered to be a buffer period to allow vaccination to take effect.

During the whole investigated period (18 months before and after vaccination), the herds from the vaccinated group had better performances (total born and weaned per litter - Table 8). However, farms from the vaccinated group had a higher frequency of low production periods (not shown) as indicated by calculated number of unproduced piglets during these periods (Table 8).

Herd vaccination with the autogenous killed virus vaccine did not improve the productivity of the vaccinated herds (Table 8). However, over the two years of the project, there was a deterioration of the health situation in the control farms (increase in the number of unproduced piglets related to low production periods: 0.56 vs. 0.44), while the situation on the “vaccinate” herds remained stable (0.63). This stability of the production of the vaccinated herds compared to the deterioration of the control herds might suggest some effect of the vaccine.

Table 8 Herd performance (mean values) in vaccinated and control herds 18 months before and after vaccination started (September 2008).

Piglets	Group (Vaccinated)		Group (Control)	
	Jan.-2007- Aug.-2008	Dec.-2008- July-2010	Jan.-2007- Aug.-2008	Dec.-2008- July-2010
	Average	Average	Average	Average
Total born per litter	12.58	12.83	12.39	12.70
Born alive per total born	0.92	0.92	0.92	0.91
Litter per breeding	0.82	0.81	0.83	0.80
Weaned per born alive	0.87	0.86	0.87	0.85
Weaned per litter	9.99	10.08	9.85	9.87
Weaned per service	8.22	8.19	8.19	7.93
Unproduced piglet* (piglet/year/sow inventory)	0.64	0.63	0.44	0.56

\* Normalized for a production of 22.62 weaned piglets /sow inventory (FPPQ, 2010)

### 3.8 Financial impact of PRRS virus outbreaks

The financial impact of PRRS virus outbreaks was assessed using the summation of the estimated unproduced piglets during the periods of low productivity identified with the indicator weaned piglets per service (see Figure 1). All the periods of low productivity are not necessarily related to PRRS outbreaks, but data analysis shows that 80% of these periods of low productivity could be related to PRRS outbreaks (Table 7).

All the economic estimates were standardized for a 600 sow unit, producing 22.62 piglets per sow per year that could be sold for \$28.98 per piglet (FPPQ, 2010). The following estimates were calculated:

- 1) Odds of having a PRRS outbreak for a sow unit located in the Beauce area is 50% per year (one outbreak every two years).
- 2) The duration of the periods of low productivity was variable but a duration of at least 16 weeks was common.
- 3) The average weekly reduction of the production during a period of low productivity (at least 80% related to PRRS outbreaks) was 29.9%.
- 4) The standard sow unit production would have a reduction of 78 piglets per week of PRRS outbreak for a total of 1 248 unproduced piglets for a typical 16 week PRRS outbreak.
- 5) These unproduced piglets would translate in a \$2,258 drop in income per week of outbreak and a total income loss of \$36,128 for a 16 week outbreak (or \$60 per sow).

All the economic impacts calculated in this project were only based on the number of unproduced piglets that are most likely linked to PRRS outbreaks. These calculations do not include all the extra costs related to the management of the PRRS outbreak and all the losses reported to occur in the nurseries and grow-finish units.

## 4 Conclusion

This project was an innovation for the participating producers (>36) and their veterinarians (>9). Indeed, participating producers accepted to share information about the circulation of PRRS virus between the participating farms, they accepted to share the PRRS viruses to allow their inclusion in the common autogenous vaccine, and, finally, they accepted to share their production data to estimate the efficiency of the vaccination. Retention rate of participating farmers in this project was very high (90% - 36/40 farms).

The vaccination strategy on a regional level with an autogenous vaccine, although a very appealing methodology to get a better immunity in order to control the Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) was not found to be an appropriate methodology for this zone. The major problem was related to the very high diversity of PRRS viruses circulating in such a small territory (20 km diameter).