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DS Infectious Animal Diseases

PRRS Biggenmonitoring

SEQUENCING AT CODA-CERVA / SCIENSANO

General data

Between February 2015 and November 2017, 618 serum samples that were tested as positive in pool of 3 samples at DGZ have been received at CODA-CERVA (now SCIENSANO) (about 300 per year in 2015 and 2016) for sequence determination (Table 1, Figure 1). About sixty percent of them have given an informative result after PCR amplification of 600bp in the ORF5 region and sequencing. Negative results were obtained either due to presence of negative serum samples in positive pool, the presence of very low amount of virus that is detected with sensitive test like real-time RT-PCR but doesn't allow amplification in conventional PCR, or the presence of multiple PRRSV isolates inside one sample that impair a correct reading of the sequence. The number of negative results in sequencing has been lowered since September 2016 after the implementation of sequencing reaction on pool instead of individual serum (Figure 1). Still this change regularly requires the separation of pools for determination of the sequence when results are not conclusive (no PCR amplification, mix of sequences) but this data are not recorded in the table below.

Table 1: Number and percentage of samples analyzed for sequencing between 2015 and 2017 for Biggenmonitoring

Year	NEGATIVE		TYPE 1 - EU		TYPE 2 - NA		Grand Total
2015	153	*49,8%*	140	*45,6%*	14	*4,6%*	307
2016	132	*38,4%*	168	*48,8%*	44	*12,8%*	344
2017	31	*27,9%*	64	*57,7%*	16	*14,4%*	111
Grand Total	316	*41,5%*	372	*48,8%*	74	*9,7%*	762

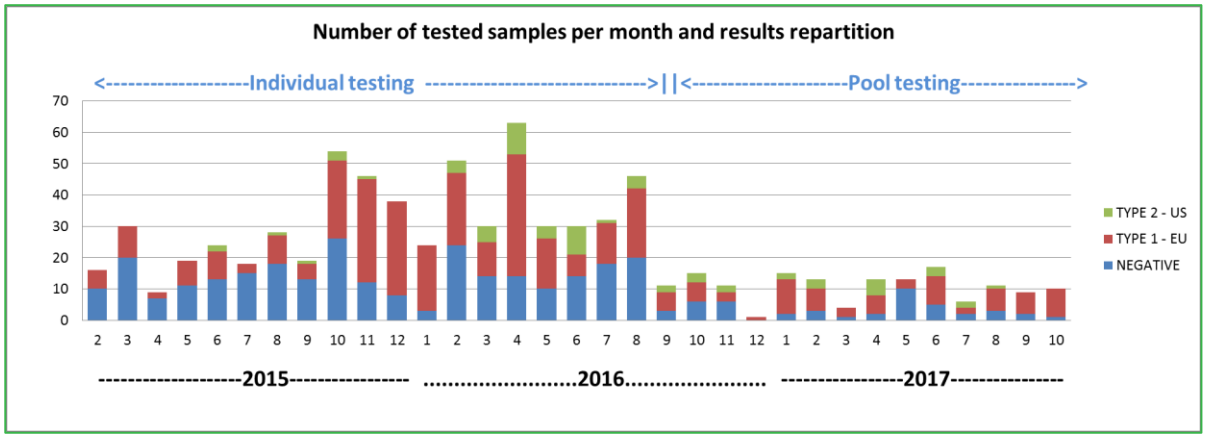

Figure 1: Number of samples tested per month in 2015, 2016 and 2017 and results repartition by genotype

Geographical dispersion

Nearly all the samples were collected in Flanders region (Figure 2). Isolates with readable sequence were identified in the different Flemish provinces, with a majority representation of Antwerp (39.6%) and West-Vlaanderen (35.8%) (Table 2) and largely distributed in the different municipalities within each province. The remain of the data originated equally from Limburg (11,9%) and Oost-Vlaanderen (12,1%).

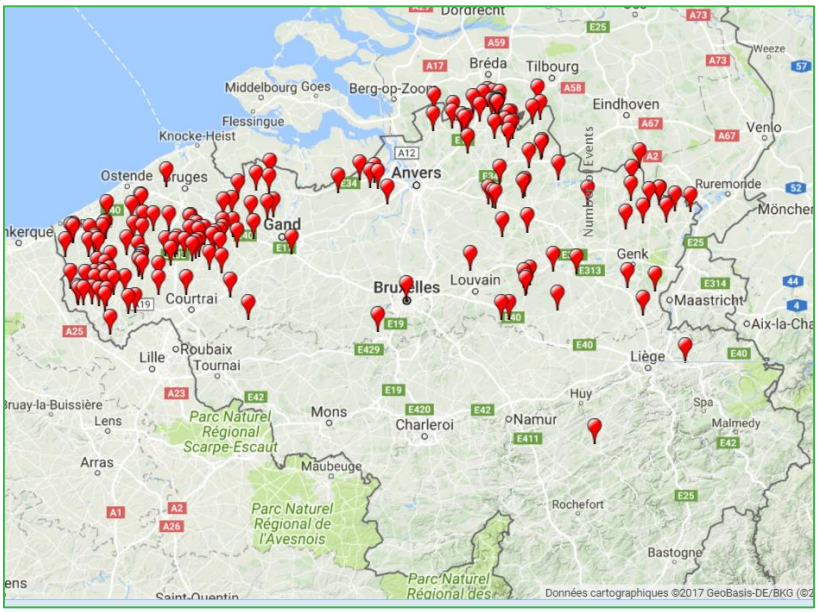

Figure 2: Localisation of the isolates collected for Biggenmonitoring (2015-2017)

Table 2: Number of municipalities with identified PRRS isolates and total number of available sequences per province between 2015 and 2017

	2015		2016		2017		Grand Total	
Province	**Municipalities**	**Nb seq**	**Municipalities**	**Nb seq**	**Municipalities**	**Nb seq**	**Nb seq**	**%**
ANTWERP	13	72	15	72	13	36	**180**	*39,6%*
BRABANT	0	0	1	1	0	0	**1**	*0,2%*
LIEGE	0	0	1	1	1	1	**2**	*0,4%*
LIMBURG	6	9	10	27	0	18	**54**	*11,9%*
OOST-VLAANDEREN	8	27	6	19	5	9	**55**	*12,1%*
WEST-VLAANDEREN	15	53	24	95	8	15	**163**	*35,8%*
Grand Total	**42**	**161**	**57**	**215**	**27**	**79**	**455***	***100%***

**The total number of sequences is slightly different from the number of sequences described in the general data (372+74=446) as it included some additional individual sequences from separated pools.*

For all the period covering 2015 to 2017, the sequences represented the PRRSV genome diversity present in 146 different herds representing essentially the provinces of Antwerp, Limburg, Oost- and West-Vlaanderen (Table 3).

Table 3: Number of herds with available PRRS sequence per province between 2015 and 2017

Number of sampled herds	2015	2016	2017	2015-2017
ANTWERP	28	28	21	45
BRABANT	0	1	0	1
LIEGE	0	1	1	2
LIMBURG	6	11	9	24
OOST-VLAANDEREN	8	6	5	16
WEST-VLAANDEREN	23	43	10	59
Grand Total	65	90	46	146

Genetic analysis

The large majority of sequences obtained in the Biggenmonitoring corresponded to Type-1 European isolates (82.2% of the total positive samples) whereas Type-2 (North American) isolates represented the others 17.8% (Table 4). There is large variation between province, with West-Vlaanderen presenting a lower than average percentage of Type-2 sequences (7,4%) whereas this genotype is more largely find in the samples collected in Antwerp, Limburg and Oost-Vlaanderen (22,8 to 27,3%).

For all the period covering 2015 to 2017, the sequences represented the PRRSV genome diversity present in 146 different herds representing essentially the provinces of Antwerp, Limburg, Oost- and West-Vlaanderen (Table 3).

Table 4: Genetic repartition of PRRS isolates from 2015 to 2017 by province

Number of sequences	2015		2016		2017		Total			
	EU	NA	EU	NA	EU	NA	EU		NA	
ANTWERP	67	5	43	29	29	7	139	*77,2%*	41	*22,8%*
BRABANT	0	0	1	0	0	0	2	*100%*	0	*0,0%*
LIEGE	0	0	1	0	1	0	2	*100%*	0	*0,0%*
LIMBURG	9	0	22	5	10	8	41	*75,9%*	13	*24,1%*
OOST-VLAANDEREN	21	6	12	7	7	2	40	*72,7%*	15	*27,3%*
WEST-VLAANDEREN	50	3	88	7	13	2	151	*92,6%*	12	*7,4%*
Grand Total	**147**	**14**	**167**	**48**	**61**	**19**	**375**	***82,2%***	**81**	***17,8%***

The phylogenetic analysis of the sequences obtained in the BiggenMonitoring was based on a Clustal Alignment (ClustalO) of nucleotide sequences, in presence of references (Lelystad, VR-2332, Lena, vaccine strains for Porcilis, Unistrain, PRRSFLEX, pMLV, Belgian and European published sequences,…) and on the algorithm for assessing the Maximum Likelihood inside the MEGA package. The genetic clustering indicated the presence of two major genotypes : The European-Type genotype, which represents the most of the sequences observed in Belgium and the North-American genotype (Figure 3).

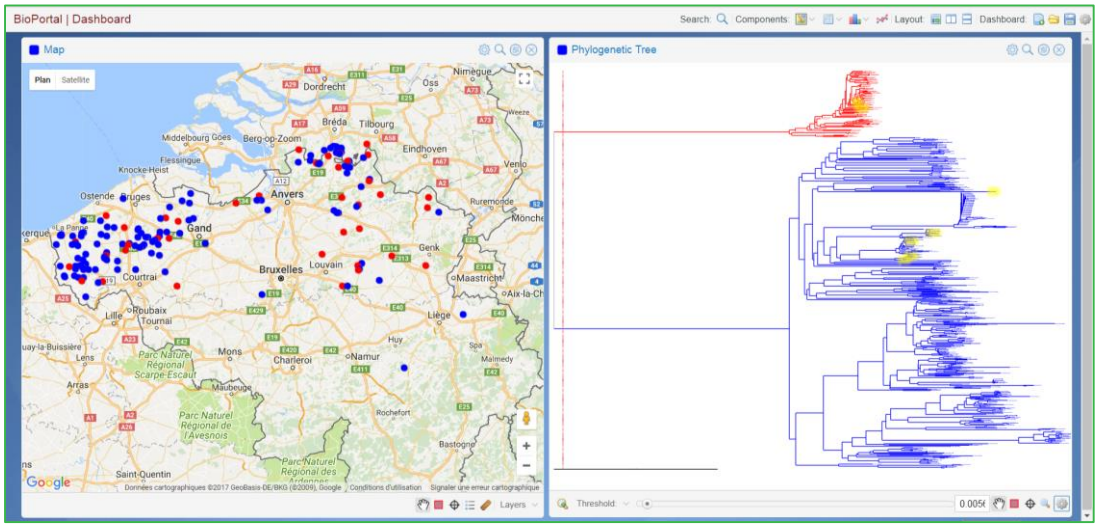

Figure 3: Geographical localization and phylogenetic clustering of PRRS isolates - Isolates clustering with Lelystad (Type-1 genotype) are colored in blue whereas those clustering with Type-2 genotype are colored in red.

In the North-American genotype subgroup (genotype 2), the sequences corresponding to the pMLV vaccine (Type 2) are present in the different provinces (Figure 4). The homology percentage to VR-2332 and pMLV is very high [min 0,958 – max 0,993] and confirms that all the isolates of Type-2 genotype observed in Belgium have a vaccine origin.

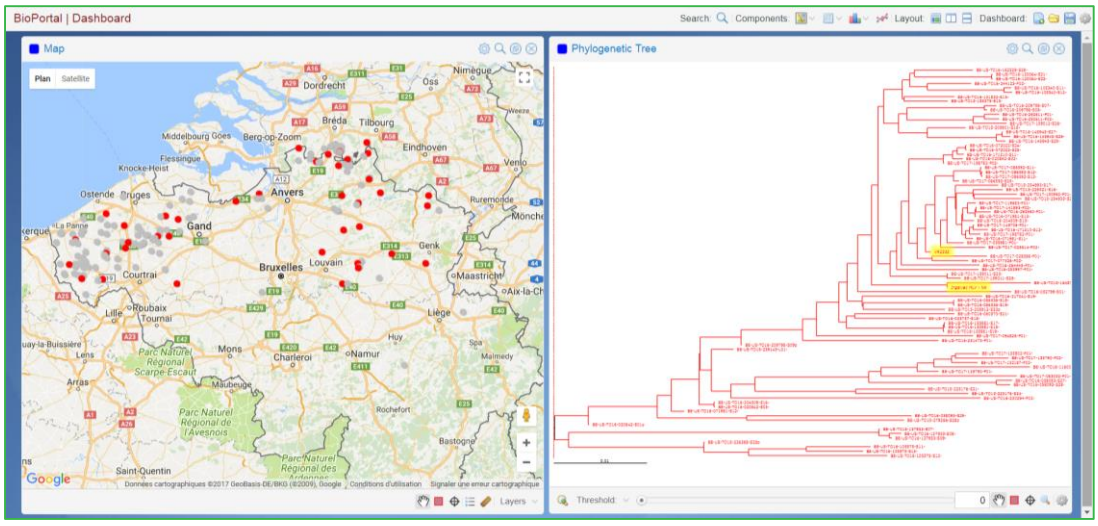

Figure 4: Geographical localization and phylogenetic clustering of PRRS Type-2 isolates - Isolates clustering with vaccine strain from Type-2 genotype are colored in red.

In the European genotype group (genotype 1), all the isolates clustered with Lelystad and belonged to genotype 1.1. (Figure 5). In this group, the general homology to Lelystad can be very variable [Min 0,731 – Max 0,988]. The isolates (n=33) presenting the highest homology to Lelystad are derived from the different vaccines (Porcilis, Unistrain, PRRSFLEX). Their homology to Lelystad varied between 0,899 and 0,988. They presented a separate cluster on the phylogenetic tree, inside the Lelystad cluster (Figure 5).
When vaccine-derived isolates are excluded the maximal homology to Lelystad for a "field" isolate is 0,902.

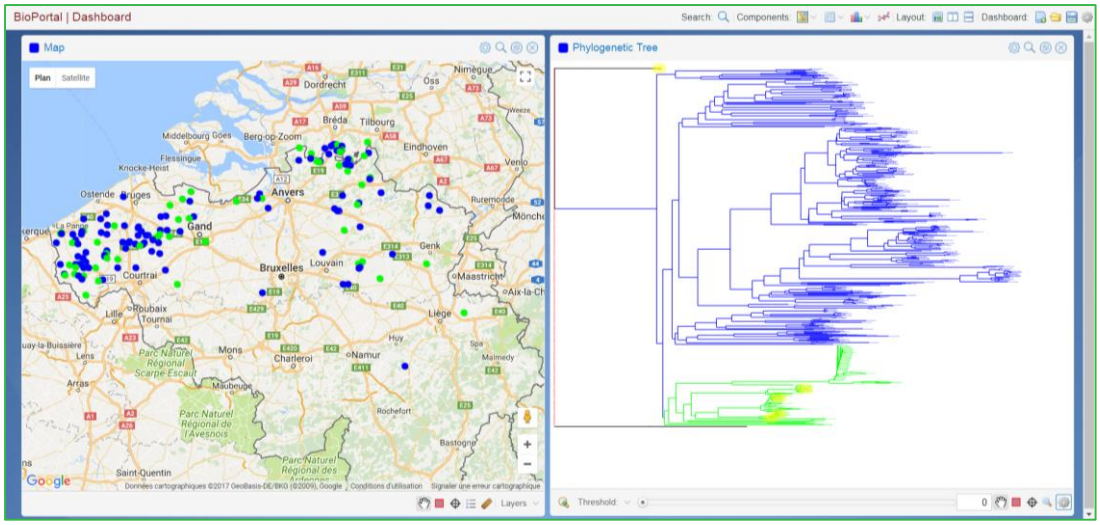

Figure 5: Geographical localization and phylogenetic clustering of PRRS Type-1 isolates – Isolates clustering with vaccine strains are colored in green whereas "field" isolates are colored in blue.

Genetic and Temporal correlation

Beside the presence of one cluster that regroups sequences that were only observed in 2015, there is no other specific segregation observed in relation with the year of collection (Figure 6-a).

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Genetic and Geographical correlation

The provinces West-Vlaanderen and Antwerp are the two that are the most represented on the phylogenetic tree (Figure 6-b). In the tree one can observed that very closely related isolates tend to be found in the same province. However there is no specific distribution related to the province of origin.

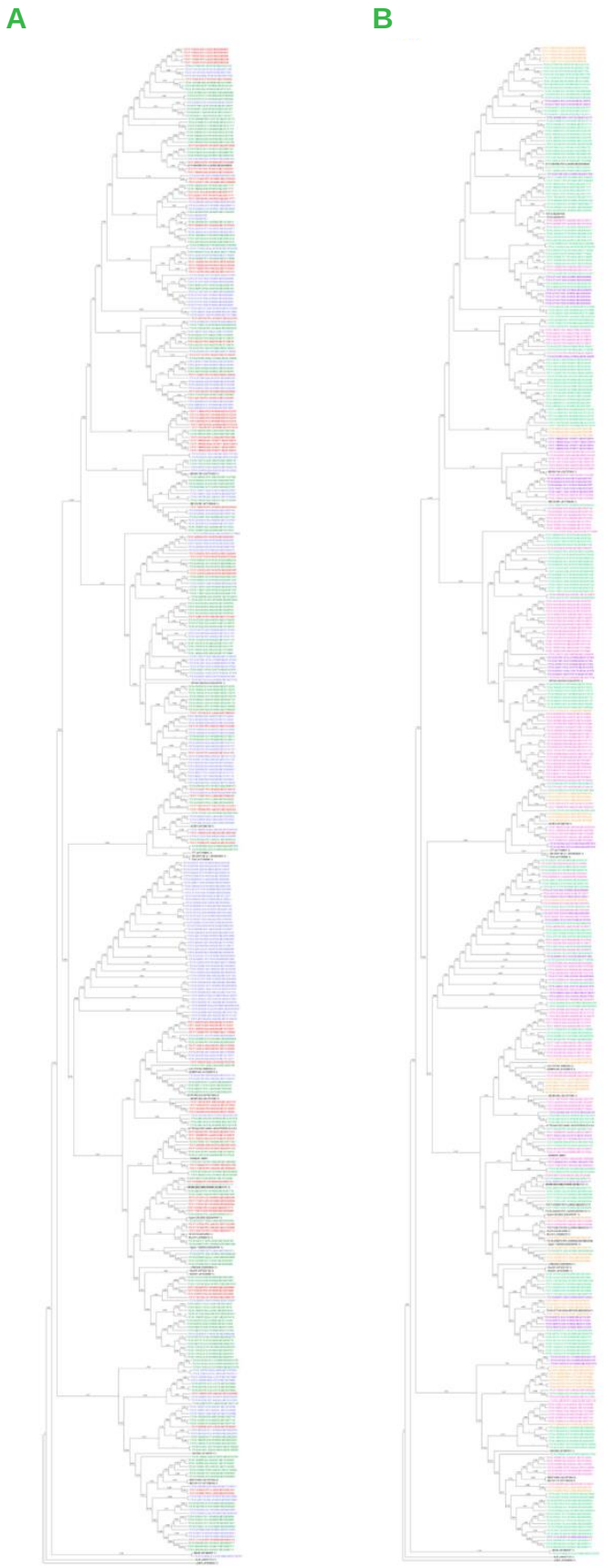

Figure 6: Phylogenetic tree of isolates from Biggenmonitoring collected during 2015-2017.
A) Isolates according to the year of sampling : from 2015 in blue, 2016 in green and 2017 in red.
B) Isolates according to the province of samping : Green in West-Vlaanderen, purple in Oost-Vlaanderen, pink in Antwerp, Orange in Limburg.

Case studies

During the biggenmonitoring the herds were sampled regularly but didn't always give positive results and sequences. In our sequence database, we have sequences corresponding to 146 herds. For 91 herds we have sequence(s) from one sampling date. For 42, 8 and 5 herds, we have sequences corresponding to two, three or four different sampling dates respectively.

By following the genetic homology between isolates from different collection dates on the same farm and their phylogenetic relationship, it is possible to determine case by case if a farm has on-going infection with one or more than one isolate or if it is going through different episodes of infection by other isolates through reintroduction.

For example, in the 5 herds with 4 different sampling dates, we have observed one case were only vaccine strain was find at the different sampling dates, two cases were both vaccines and one field strain were observed, one case with vaccine strain and two different field strains on different times and one case with one field strain that remains present through the three years of program (sampling dates in 2015, 2016 and 2017).