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Real-Time PCR Diagnostic Test of Rat Parvovirus Infections and Genetic Strain Identification – Comparative study with serological patterns

SUMMARY

The Rodent Parvovirus group currently includes four different virus types : Kilham Rat Virus (KRV), Toolan's H1 (H1), Rat Minute Virus (RMV) and Rat ParvoVirus (RPV). The virus type involved in an infection must be determined to assess clinical consequences and possible interferences on scientific studies. As there are cross-reactions in serologic assays, it is difficult to type the virus, especially for recently characterized RPV and RMV. However, the association of Real-Time PCR technology and genetic typing by sequencing offers a sensitive and specific diagnostic, especially useful to ascertain seropositive results and to solve inconclusive serologic pattern. Furthermore, acute strain identification allows contaminations monitoring in facilities.

INTRODUCTION

The serological screening for Parvovirus infections in rat, has not been quite satisfactory for few years. Mainly, it does not frequently allow to ascertain the virus type, especially for the newly recognized ones. However, this identification may have a major importance, because of their different clinical consequences and interferences with experimental results.

In this study, 38 batches of sentinel rats have been tested, both with serological and molecular biology assays. The comparison of these results is shown hereafter.

MATERIALS AND METHODS

PCR (Scanelis)

Nucleic acids extraction from Rat Caeca Samples (2 to 4 caeca pooled)
Protocol of « High Pure PCR Template Purification Kit », Roche®

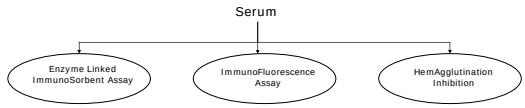
Rodent Parvovirus specific Real-Time PCR

Negative Result — **Positive Result**

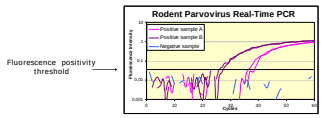
PCR and Sequencing (162 bp)
Sequence analysis, comparison with sequence of reference strains and others strains from the same facility or institution

Serology (Vebiotel and subcontractors)

- Sampling of caeca and sera was performed by Vebiotel Laboratory for each tested animal
- Serological analyses were performed in QM Diagnostics (The Netherlands) and CRF Laboratories (France)
- Vebiotel analyzed every serological pattern and gave conclusions or hypothesis



RESULTS 11 positive batches, 2 of them (from 1 institution) infected with KRV and 9 (from 3 institutions) with RMV type



Rodent Parvovirus Real-Time PCR test in Scanelis

- no +/- result and **a semi-quantitative approach**: sample B has a higher viral load than sample A (about 1000 times more)
- a rodent parvovirus **specific** and **sensitive** test

Batches serological positive results compared with molecular typing results in four different institutions

Batch N°	Sample N°	Facility	KRV ELISA	KRV HAI	KRV IF	H1 ELISA	Serological findings	PCR and sequencing typing (if performed)
1	1a	B	+	1/20	+	+	Parvovirus ? Seroconversion beginning ?	KRV
	1b		-	-	+/-	-		
	1c		-		-	-		KRV
	1d		-					
2	2a	A2	+	-	+	+/-	RMV ?	RMV
	2b		-	-	+	-		
	2c		+		+	+/-		positive
	2d		+/-		+	-		
3	3a	A2	+		+	+/-	RMV ?	RMV
	3b		+/-		+	+/-		
	3c		-		+	-		
	3d		-	-	+	-		
	3e		+	-	+	+/-		positive
	3f		+/-	-	+	+/-		
4	4a	C	+	-	+	+	KRV ? RMV ? Both ?	RMV
	4b		+	> 1/80	+	-		RMV
	4c		+	-	+	+		RMV
5	5a	A2	-		-	-	RMV ?	negative
	5b		-		-	-		
	5c		+	-	+	+		RMV
	5d		+	-	+	+		
6	6a	A2	+	-	+	+	KRV ? RMV ? Both ?	RMV
	6b		-	-	+	-		
	6c		+	-	+	+		RMV
	6d		+	1/80	+	+		
7	7a	A2	+	1/40	+	+	RMV ?	positive
	7b		+	-	+	+		
	7c		+	-	+	+		positive
	7d		+	-	+	+		
8	8a	A1	-	-	-	-	RMV ?	negative
	8b		-	-	-	-		
	8c		+	-	+	+		RMV
	8d		+	-	+	+		
9	9a	A1	+/-	-	+	+/-	RMV ?	RMV
	9b		+	-	+	+/-		
	9c		+	-	+	+		positive
	9d		+	-	+	+		
10	10a	D	+	1/20	+	+	RMV ?	positive
	10b		+	-	+	+		RMV
	10c		+	1/20	+	+		positive
11	11a	D	-	-	+/-	-	Seroconversion beginning ?	negative
	11b		-	-	-	-		negative

	Seropositive	Seronegative
PCR positive	11	0
PCR negative	1 +/- sero	26

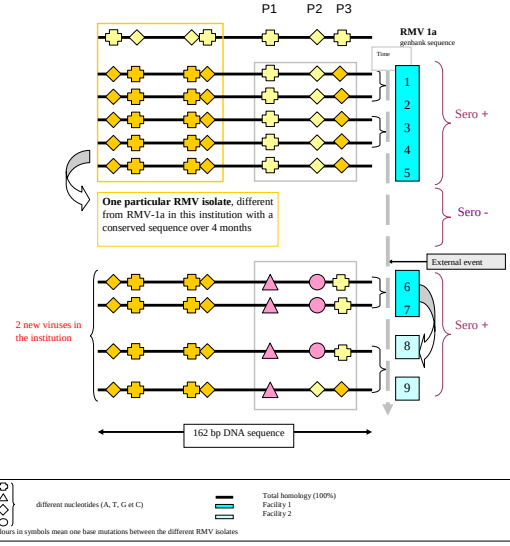
Serological versus PCR result for each batch

Furthermore, a 2 pooled caeca sample was positive in PCR whereas the 2 individual serological results are negative.

- PCR and serological results provide a good agreement for one batch
- Whereas the **serological patterns inconsistently allow viral identification**, the association of **PCR and sequencing provides the Rat Parvovirus type.**
- According to these results, a limited **number of sentinels** may be tested with PCR assay
- PCR enables to confirm or infirm the infection, when serological patterns are inconclusive

Comparative Analysis of RMV isolates DNA sequences in one institution:

Genetic stability of a Rat Minute Virus isolate during a relative long period in a same facility and **Rat Minute Virus** typing in a same institution for contamination monitoring



- 100% homology over 4 months seems to mean **a relative genetic stability** of this RMV isolate
- Molecular typing: a tool for the **approach to the RMV's strain** The variability of isolates in a same institution helps to implement for contamination management
- The 162 pb product sequencing is sufficient to determine the Rat Parvovirus Type

However, it would be interesting to sequence a longer fragment of the viral genome, to obtain a more accurate identification of the viral isolate. It may improve the contamination monitoring in an institution

CONCLUSIONS

- **Serological and PCR assays are complementary for an early and precise diagnosis of parvoviral infections**
- **This molecular assay allows :**
  - **an accurate diagnosis and typing of the parvovirus species :** KRV, Toolan H1, RPV (RPV-1a) and RMV (RPV2)
  - **confirmation or infirmation of an inconclusive serological pattern**

Perspectives:

- Environmental monitoring
- Screening in non-immunocompetent rats
- Testing others samples may enhance sensitivity (mesenteric lymph node) or allow diagnosis on live animal (faeces) ? Pooling samples may reduce analysis cost