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

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

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

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

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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 a relative genetic stability

One particular RMV isolate: Different RMV-1a at this institution with conserved sequence over 4 months

P1 | P2 | P3 | RMV 1 | 2 | 3 |

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 inflm the infection, when serological patterns are inconclusive

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 inflmation of an inconclusive serological pattern

Perspectives:

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

SUMMARY

The Rat 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 serological assays, it is difficult to type the virus.

The variability of isolates in a same institution helps to implement for contamination management. 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.