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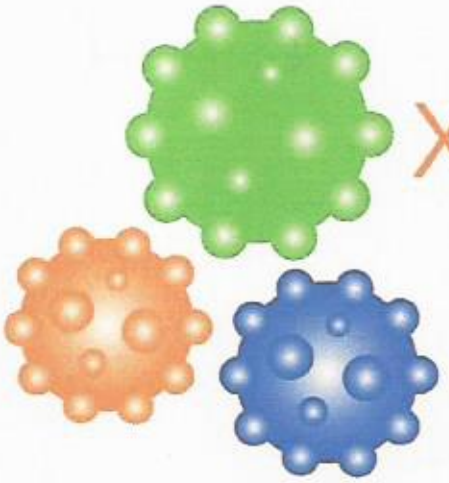

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Changing Viruses in a Changing World

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A cell culture-adapted Classical swine fever virus phenotype does not require the 476Arg Erns mutation

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Classical swine fever virus (CSFV) is a small, enveloped RNA virus of the genus *Pestivirus* within the *Flaviviridae* family, and previous studies gave further insights into cell – virus- interactions of CSFV.

It was e.g. demonstrated that the envelope glycoproteins E^{rns} and E2 play the major role for virus attachment and entry. While E^{rns} mediates the initial contact to the host cells, binds E2 the specific receptor, porcine complement regulatory protein CD46. It is known that in vitro propagation of CSFV leads to cell culture-adapted phenotypes that are characterized by superior infection rates and viral growth in vitro. These virus variants are able to use cellular Heparan sulfate (HS) as a complementary receptor to attach to the host cells. It was described for CSFV strain "Brescia" that a single amino acid change in the envelope protein E^{rns} (aa 476 Ser to Arg) is responsible for the HS binding. DSTP-27 a N,N`bisheteryl derivate of dispirotripiperazine was shown to bind to cellular heparan sulfates, and is thus able to inhibit infection of cell culture- adapted virus variants of CSFV.

In the framework of recent receptor studies, field-type and cell culture-adapted variants of the CSFV strain "Roesrath" were investigated. The cell culture-adapted phenotype was represented by the 50th cell culture passage. The adaptation was proven by a strong influence of HS-binding compound DSTP-27 that was able to block infection to a very high extent. Despite the obvious phenotype, partial and full- length sequencing showed that the observed ability to bind HS after passaging was not accomplished through an expected amino acid exchange in the E^{rns} at amino acid position 476. The original field strain as well as the 50th passage of the virus still presented a Serin at this position. Instead of the expected mutation, the propagated virus variants showed an exchange from Asp to Asn in the E^{rns} (aa355) and from Leu to Ser in the glycoprotein E2 (aa763).

In order to investigate the impact of these changes, the point mutations, leading to both amino acid exchanges, were integrated into the consensus sequence of the original field variant of CSFV "Roesrath" (GenBank entry: GU233734) with the help of a pBelo BAC reverse genetics system. The resulting clones are under investigation with regard to growth kinetics, receptor usage and mutation rate.

Irrespective of the final outcome of our studies regarding the impact of the described mutations in the E^{rns} and E2 of CSFV, investigations of the alternative way of cell culture adaption will shed light on the infection process of CSFV in vitro.

Next-generation sequencing fails to identify viral miRNAs encoded by PCV2 in subclinically infected pigs

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Objective: MicroRNAs (miRNAs) are small non- coding RNAs with post- transcriptional regulation functions. These small RNAs are expressed by a huge variety of organisms, from mammals, to plants and recently discovered, viruses. Within the viruses, it has been demonstrated that some DNA viruses are capable of express miRNAs. PCV2 is a small single stranded DNA virus with 1.76 Kb and is the aethiological agent of post weaning multisystemic wasting syndrome. In this study, small RNA libraries were constructed from two tissues of subclinically PCV2 infected pigs to explore if PCV2 can encode viral miRNAs.

Methods: Firstly, *in silico* prediction was carried out in order to check if the PCV2 genome encodes possible miRNA precursors by using Vmir software. Then, four animals were inoculated with $7x10^{4.8}TCID_{50}$ of PCV2 iso-