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Conclusion: The method described above enables our laboratory to obtain clean MLVA profiles rapidly for data entry into the MLVA database. This will also help reduce time taken to identification and determining if a particular outbreak is due to a point source contamination. In addition, we hope other researchers who work with *Vibrio cholerae* would consider contributing to this public MLVA database to allow for building up of a truely useful public resource.

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Neutralizing antibodies against pandemic, seasonal and avianlike H1N1 swine influenza virus in swine contacts and swine, Western-Europe

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Background: Serological studies for swine influenza viruses (SIVs) in humans with occupational exposure to pigs have only been reported from the Americas, but not from Europe. Human infections with SIV have been rare and mostly reported in contacts with swine, as serological studies suggest that swine workers are at increased risk of zoonotic infection with SIV.

Methods: Antibodies against pandemic H1N1 2009 influenza virus, a 2007/2008 seasonal H1N1 virus and an avian-like, enzootic H1N1 SIV were analyzed by virus neutralization assay in 211 swine contacts in Luxembourg compared to a matched general population. In addition, neutralizing antibodies to SIV and H1N1 2009 in 203 swine were determined.

Results: We showed that swine workers have more often and higher titers of antibodies against pandemic flu and SIV than the control population, and that these titers cannot be explained by cross-reactivity with antibodies at least from recent seasonal flu. Antibodies against SIV and pandemic H1N1 2009 correlated with each other but not with the 2007/2008 seasonal H1N1. The pigs showed a strong positive antibody response to avian like H1N1 and to a weaker extend to H1N1 2009. A correlation between the two virus subtypes was observed, but not to the same extend as for the human cohort.

Conclusion: Higher antibody titers against the pandemic H1N1 2009 virus and the avian like SIVs were found in swine workers compared to the general population. As this cannot be explained by cross reactivity from recent seasonal viruses only, we assume that in SIV antibodies swine workers recognize an epitope that is also present in pandemic H1N1 2009 virus, but that is not widely recognized by antibodies found among the general population.

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Deep sequencing of H1N1pdm Influenza A clinical samples from Luxembourg 2010/2011

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Background: Surveillance of Influenza A Virus (IAV) is crucial to heighten the preparedness for a next IAV pandemic. In this study, we analyzed clinical IAV samples by next generation sequencing (NGS) to allow detection of minor frequency reassortments and/or SNPs, which can lead to antiviral resistance or increased pathogenicity in human strains even before they become manifest.

Methods: We investigated 41 clinical IAV H1N1 samples from Luxembourg (season 2010/2011) by next generation sequencing on a Roche GS Junior. Samples were studied for HA and NA by amplicon sequencing. In total, 147 285 sequences were analyzed.

Results: Overall, 172 different SNPs could be identified: 44 variants for HA and 21 variants for NA resulted in amino acid changes. None of the NA sequences showed the H274Y or I223R mutations that are associated with drug resistance against oseltamivir or zanamivir. Eight low frequency variants of the 21 found in NA were not described before. A variant found in nearly all samples (25/27) in a high frequency (99-100%) was V241I, which is also found in 39% of H1N1 sequences worldwide.

Three of 30 samples showed a high frequency (86,5-100%) amino acid change in the HA highly conserved epitope region 2. One sample showed an additional amino acid change in the same epitope in a high frequency (27%). Both amino acid changes together lead to a change in the predicted secondary structure. Several low or high frequency variants in the receptor binding site and in the highly variable regions could in part also change the predicted secondary structure of the HA.

The potential impact of these major and minor variants will be further discussed.

Conclusion: Taken together, this study shows that investigation of clinical IAV samples by NGS allows detection of minor to moderate frequent IAV variants and provides in depth insight in the molecular evolution of IAV on a population level.

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