genome of pigs. Whereas PERV-A and PERV-B are present in all pigs and infect human cells in vitro, PERV-C infect only pig cells and is not present in all pigs. In addition, recombinant PERV-A/C infecting human cells and replicating at a higher rate than the parental PERV-A were found. Among all investigated pig strains Göttingen minipigs belong to the best characterized. Göttingen minipigs were established at the University of Göttingen, using the founder breeds Minnesota minipigs, Vietnamese potbelly pig and German landrace. The animals bred at Ellegaard, Denmark, are screened regularly for the absence of 20 bacteria, 17 viruses, three fungi and four parasites. Since the herd is produced in full-barrier specified pathogen free facilities and their physiologic parameters, their health status and genetics are well-defined, Göttingen minipigs are used worldwide for biomedical research. We analysed for the first time the presence and expression of PERVs in these animals using PCR and real-time PCR methods. PERV-A, PERV-B, PERV-C, but no recombinant PERV-A/Cs were found in the genome of all investigated Göttingen minipigs. Expression of PERV was compared with that in previously analyzed pig strains. It was slightly higher than in German landrace and some other breeds, but lower than in Yucatan miniature pigs. Virus particles able to infected human 293 cells were not detected even after mitogen treatment of the PBMCs which stimulates expression of endogenous retroviruses. In addition, 7 other bacteria, four viruses, two fungi and two parasites were tested negative. Using a RT-PCR and real-time PCR hepatitis E virus was not detected in liver and kidney of minipigs.

To summarise, since the Göttingen minipigs are well-characterised, are free of potential zoonotic microorganisms and characterized by low expression of PERV, they may be considered as good candidate animals for islet cell xenotransplantation.

Zoonoses and Emerging Viruses

Poster-Nr: 085

Abstract-ID: 380

The effect of S.suis co-infection on the infection of well-differentiated porcine respiratory epithelial cells by swine influenza viruses

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Disease often occurs due to a combination of various factors including viral and bacterial pathogens as well as environmental factors. Amajor factor responsible for severe virus infections may be bacterial co-infections. As known, pigs are important hosts for influenza A viruses and may play an important role in the interspecies transmission of influenza viruses. Primary target cells for Influenza viruses are the epithelial cells in the respiratory tract. Differentiated airway epithelial cells contain special cell types such as ciliated cells or mucus-producing cells that can not be maintained as immortalized cell cultures. We have recently reported a culture system for differentiated respiratory epithelial cells to analyze the infection of porcine influenza viruses in their natural target cells. Therefore, the aims of this study are to analyze the effect of *S.suis* co-infection on the infection of well-differentiated porcine respiratory epithelial cells by porcine influenza virus types H1N1 and H3N2. Specifically, infection will be analyzed by cells pre-infected with influenza virus. The comparison will reveal to what extent the bacterial infection enhances the severity of infection by porcine influenza virus. We compared five porcine viruses of the three subtypes currently prevalent in the swine populations (H3N2, H1N1, H1N2) with respect to the following parameters: (1) duration of the growth cycle; (2) amount of infectious virus released into the supernatant; (3) extent of the ciliostatic effect. These viruses showed differences in their growth behavior and ciliostatic effect on PCLS and thus reflected the virulence properties of these viruses. Our co-infection studies will reveal whether *S.suis* differentially affects influenza viruses differing in their virulence.

Zoonoses and Emerging Viruses

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Abstract-ID: 386

The adaptation of avian influenza viruses to the respiratory epithelium of pigs

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Pigs are an important host for influenza A viruses and may play a crucial role in the interspecies transmission. To analyze the infection by influenza viruses, we have established precision-cut lung slices from the porcine lung as a culture system for differentiated respiratory epithelial cells. In precision-cut lung slices, the differentiated epithelial cells are maintained in their original setting. As differentiated repiratory epithelial cells are the primary target cells for influenza virus infections, precision-cut lung slices provide an interesting system to analyze the adaptation of avian influenza viruses to the respiratory epithelium of pigs. Avian influenza viruses H9N2 subtype have been circulating worldwide in multiple avian species and have repeatedly infected mammalian to cause typical disease. The continued avian-to-mammalian interspecies transmission of H9N2 viruses raises concerns about the possibility of viral adaption with increased virulence for humans and poses a potential health risk to the public. Avian influenza viruses H9N2 subtype were subjected to several passages in precision-cut lung slices. Then the changes in the viral properties that are associated with the adaptation process were characterized by analyzing: (1) duration of the growth cycle; (2) amount of infectious

virus released into the supernatant; (3) extent of the ciliostatic effect. Adaptation of the avian viruses to growth in porcine cells was evident in a shortening of the growth cycle. Sequence analysis revealed that few amino acid changes occurred during the different virus passages. The importance of the individual mutations is currently analyzed by generating recombinant viruses that contain the respective mutated proteins. Our study will help to understand the processes involved in the adaptation of H9N2 influenza viruses to new hosts.

Zoonoses and Emerging Viruses

Poster-Nr: 087

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Streptococcus suis affects the replication of swine influenza virus in porcine tracheal cells.

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Influenza viruses are important pathogens in pig farms causing infections that are associated with large economic losses. Surveillance results show that swine influenza viruses (SIV) were found in 31.36% of European pig farms. *Streptococcus suis* is one of the most important bacterial respiratory pathogen in the swine population. Secondary infection by *S. suis* may enhance the severity of disease in piglets infected by SIV, but the relationship between SIV and *S. suis* remains unclear. Here we established an *in vitro* co-infection model for SIV and *S. suis* based on newborn pig trachea cells (NPTr). Different SIV variants A/sw/Bad Griesbach/IDT5604/2006 H1N1 and A/sw/Herford/IDT5932/2007 H3N2 were used. Our recent studies showed this H1N1 strain only had a mild effect on the ciliary activity, while the H3N2 strain showed a higher replication rate and a strong ciliostatic effect in pig precision-cut lung slices. Wild type *S. suis* serotype 2 strain 10 (Wt) and an uncapsulated mutant strain (Δcps) were used as secondary infectious pathogens in this study. NPTr were infected with different combinations, first inoculated with SIV, followed by bacterial inoculation. Virus titers were measured on 3, 8, 24, 48 and 72 hour post SIV infection. Results showed the replication rates of SIV H1N1 and H3N2 were reduced in cells co-infected by *S. suis* Wt strain. The virus titers in Wt co-infected groups were ten-fold lower than in SIV mono-infection or Δcps co-infected groups at 24 h.p.i. Also, using immunofluorescence analysis, we determined the amount of bacterial adherence and colocalization of infected pathogens. At 8 h.p.i., the Wt bacterial adhesion rate was increased. Interestingly, the maximum of Wt bacterial adhesion was observed when the cells were also infected by SIV. These results indicated that *S. suis* and SIV affect each other in the infectious behavior in our NPTr cell model.

Zoonoses and Emerging Viruses

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Bats as hantavirus hosts in Africa

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Due to their ability to fly, long life span, and great population densities, bats enable efficient pathogen maintenance, evolution, and spread. Therefore, they are recognized as one of the most important reservoir hosts for emerging human pathogens. Hantaviruses (genus *Hantavirus*, family *Bunyaviridae*), mainly considered as rodent-borne viruses, were very recently found in African bats, too; Magboi virus was identified in a slit-faced bat (*Nycteris hispida*) from Sierra Leone and Mouyassué virus in a banana pipistrelle (*Neoromicia nanus*) in Côte d'Ivoire. Here we report on the detection of a new hantavirus in Noack's roundleaf bat (*Hipposideros ruber*).

Within the study, blood samples from 320 bats (137 *H. gigas*, 123 *H. ruber*, 60 *Miniopterus inflatus*) trapped near the city of Makokou, Gabon, were tested for the presence of hantavirus RNA. By using our genus-reactive nested RT-PCR screening assay targeting the large (L) genomic segment, a single positive sample, designated GB303, from *H. ruber* was obtained. To obtain more sequence data for the novel virus, we applied next-generation-sequencing approach (Illumina Miseq technology). Phylogenetic analyses of the partial L segment sequence indicated that GB303 represents a novel distinct hantavirus, provisionally called Makokou virus (MAKV). It belongs to the newly recognized, highly divergent phylogenetic group containing hantaviruses recently identified in shrews, moles, as well as bats. Magboi virus from Sierra Leone is the most closely related virus.

In addition, a quantitative RT-PCR assay was established to determine organ tropism of the virus in the infected bat. The virus could be detected in all of the available organs (brain, gut, heart, kidney, liver, spleen) while the highest virus load was observed in spleen, kidney and heart resembling hantavirus organ distribution in other reservoir hosts.

Altogether, identification of a third distinct hantavirus in African bats and its organ distribution in the infected bat further support the emerging concept of bats as yet overlooked hantavirus reservoir hosts. The impact of the newly recognized bat-borne hantaviruses on public health remains to be determined.