VETBIONET

Veterinary Biocontained facility Network for excellence in animal infectiology research and experimentation

Deliverable D7.6

Novel avian/mammalian host models for airborne transmission and susceptibility

Due date of deliverable: M66

Actual submission date: M72

Start date of the project: March 1st, 2017 **Duration:** 72 months

Organisation name of lead contractor: WBVR

Revision: V1

Dissemination level	
Public	**x**
Confidential, only for members of the consortium (including Commission Services)	
Classified, as referred to in Commission Decision 2001/844/EC	

Table of contents

1 Summary 3

2 Introduction 4

3 Results 5

3.1 Improved quantification of pathogen spread in the air (EMC) 5

3.2 Impact of bio-environment and post-natal colostrum deprivation on immunity and disease susceptibility for mono- and combined infections with PRRSV and S. suis in pigs (WBVR) 6

Study design and methods 6

Results 7

3.3 Avian influenza virus infection in chickens, Pekin ducks and mallard ducks (FLI) 11

3.4 Avian influenza virus infection and transmission in turkeys and quail (PIWET) 13

4 Conclusions 14

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014

1 Summary

Objectives

Deliverable D7.6 reports the Joint Research Activities conducted in WP7, Task 7.3 “Airborne infectious disease models” and the results obtained by the participants. The models reported here were conceived to improve the current knowledge on respiratory viral disease development and transmission in mammalian (ferrets, swine) or avian (chickens, ducks, turkeys, quail) species. The viral diseases studied include influenza (influenza A virus infection), COVID-19 and porcine reproductive and respiratory syndrome (PRRS).

Rationale

To address the various facets of respiratory infectious diseases in animal studies the experimental modalities need to be adapted to the specific research questions. One approach in Task 7.3 was directed towards the improvement of the current knowledge on the transmission of airborne viral pathogens (EMC). Tools allowing to quantify pathogen load in the air during the transmission process are difficult to set up and frequently lacking, although of high value for the interpretation of transmission studies and biological behaviour of the respective pathogen. Therefore, a novel tool for use in infection studies to assess airborne transmission of influenza virus was established. In a ferret study, infectious viral particles in exhaled respiratory droplets were captured and quantified. As a response to the COVID-19 pandemic, the ferret model was set-up as a model for SARS-CoV-2 (SARS-CoV) transmission via contact and via the air.

In experimental infection studies results regarding the host response and the course of infection can be confounded by the animal species used (for example influenza infection in mice, pigs or ferrets), the microbial background of the used animals (conventional, specific pathogen-free or germ-free) or the animals’ immune status. In a second approach in Task 7.3, differences in respiratory infectious disease development under the influence of post-natal immune development and bio-environment were examined (WBVR). The role of post-natal rearing conditions, such as colostrum-intake and exposure to a specific, microbial bio-environment was addressed by studying virus/bacterial co-infections in conventionally raised versus caesarean-derived, colostrum-deprived (CDCD) pigs, using two relevant porcine pathogens, porcine reproductive and respiratory syndrome virus (PPRSV) and *Streptococcus suis (S. suis)*.

Influenza virus transmission and infection outcomes in wild or captive birds is largely dependent on the avian host species. To take account of these inter-species differences, it is therefore necessary to develop robust animal infection models for a wide variety of avian species, notably domestic poultry species. In two complementary approaches in Task 7.3,

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement N°731014

animal infection models were set up to study and compare influenza virus infection and transmission in chickens, Pekin ducks and mallard ducks (FLI) or turkeys and quail (PIWET).

Partners involved

The partners conducting Joint Research Activities reported in WP7 Task 7.3 were: EMC, WBVR, FLI and PIWET.

2 Introduction

To address the various facets of respiratory infectious diseases in animal experimental studies the experimental modalities need to be adapted to the specific research questions. The animal models studied Task 7.3 were designed to either improve the current knowledge on the spread of airborne viral pathogens and or to examine and characterize new models for research on respiratory infectious disease development with a particular focus on the role of post-natal immune development and the microbial environment. Airborne transmission is a relevant cause of respiratory infections like for example of influenza virus infections in humans, farm animals and wild life species. Current models study transmission by contact between infected donors and non-infected recipients and can demonstrate the transfer of infectious pathogens through the air stream. However, models which allow to quantify pathogen load in the air during transmission process are difficult and frequently lacking, although of high value for the interpretation of transmission studies. Therefore, a novel tool for use in infection studies to assess airborne transmission of influenza virus was established (EMC). In a ferret study, infectious viral particles in exhaled respiratory droplets were captured and quantified. Aerosol dispensing devices were used in the optimized ferret model for airborne transmission of influenza viruses to enable standardized aerosol infection experiments without the use of infected “donor” animals. As a response to the COVID-19 pandemic, the ferret model was set-up as a model for SARS-CoV-2 transmission via contact and via the air.

In experimental infection studies results regarding the host response and the course of infection can be confounded by the animal species used, the sanitary status of the animals or factors affecting the animals’ immunocompetence, e.g. the lack of colostrum supply after birth. Such circumstances can affect the host susceptibility to infection with viruses including influenza virus and Porcine Reproductive and Respiratory Syndrome virus (PRRSV) and they can also predispose to viral/bacterial co-infections. These issues were addressed by studying viral/bacterial co-infections in conventionally raised versus caesarean-derived, colostrum-deprived (CDCD) pigs (WBVR). Special emphasis was put on assessing the status and changes in the airway microbiome in conventional and CDCD pigs before and after viral or bacterial infection (NB: the microbiome results are not reported in this deliverable, but in deliverable D8.5).

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement N°731014

Avian influenza viruses (AIV, influenza A virus) pose a constant risk to bird populations, either wild or captive. In a periodic manner single AIV gain zoonotic potential and the ability to cause spill-over infections in mammalian species (e.g. swine, mink, seals) that, in a worst-case scenario, can reach panzootic or even pandemic dimensions. Eventually human infections and deaths may result from various AIV subtypes. A particular focus in Task 7.3 was placed on developing and refining animal models for recently or presently circulating AIV in a high-containment setting. Different gallinaceous poultry (chickens, turkeys, quail) and waterfowl (Pekin ducks, mallard ducks) infection models were used to study and compare AIV transmission and infection outcomes in a variety of susceptible captive birds (FLI, PIWET).

3 Results

3.1 Improved quantification of pathogen spread in the air (EMC)

To gain more quantitative knowledge on respiratory virus transmission, and to ultimately, at least partly, replace animal studies for transmission research, air samplers were first developed or improved and subsequently tested for their efficiency to collect (non-)infectious respiratory viruses from the air (Kutter et al., 2021, Indoor Air, https://doi.org/10.1111/ina.12875). Two out of three air samplers were able to efficiently collect viruses that were artificially aerosolized in the air. One of these optimized air samplers was subsequently used in a hospital setting, to sample air around children that were admitted to the hospital with an RSV infection (Kutter et al., 2021, ARIC - not funded by VetBioNet).

Next, one of the air samplers (impinger) was used in combination with an in-house designed, 3D-printed circular tube in which aerosol-containing air is circulated by a small ventilator to prevent deposition of aerosols on the tube walls by gravity. Several influenza viruses isolated form different host species were aerosolized in this tube, circulated for multiple pre-defined time-points and subsequently collected, after which the amount of infectious and non-infectious virus was determined by virus titration and quantitative PCR (manuscript in preparation). This experimental set-up was adapted in an applied study as a response to the shortage of masks at the beginning of the COVID-19 pandemic. Several newly designed face masks were evaluated in this set-up by aerosolizing a murine coronavirus, followed by filtering with the face mask filter material and collection by an air sampler (Boogaard et al., 2021, AAQR, https://doi.org/10.4209/aaqr.2020.07.0424).

As a response to the COVID-19 pandemic, the ferret model was set-up as a model for SARS-CoV-2 transmission via contact and via the air (Richard et al., 2020, Nat Commun, https://doi.org/10.1038%2Fs41467-020-17367-2). In follow-up research, a new experimental set-up was designed and build to study the transmission of SARS-CoV-2, SARS-CoV and pH1N1 influenza virus transmission over a distance of more than one meter (Kutter et al., 2021, Nat Commun, https://doi.org/10.1038%2Fs41467-021-21918-6). This research showed for the

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014

first time that SARS-CoV-2 could remain infectious while being transported through the air in aerosols or droplets over one-meter distance. Our experience with these models was subsequently used in review papers on the use and need for animal models for SARS-CoV-2 research (Munoz-Fontela et al., 2020, Nature; Genzel et al., Curr Biol, 2022; De Vries et al., 2021 Curr Opin Virol).

More recently, quantitative data was obtained on the kinetics of influenza virus shedding, by collecting quantitative data on the amount of infectious virus particle shedding in the air. Five different air samplers and the 'influenza virus tunnel' containing electrostatic dust collectors and cell culture plates were used to sample air around virus-infected ferrets. Although infectious virus could not be collected for SARS-CoV-2, infectious pH1N1 and H3N2 influenza virus was collected for about 48 hours, after which the infectivity of the exhaled virus decreased rapidly. Additional studies are currently ongoing to study the mechanism behind this sudden decrease in infectivity.

3.2 Impact of bio-environment and post-natal colostrum deprivation on immunity and disease susceptibility for mono- and combined infections with PRRSV and S. suis in pigs (WBVR)

Study design and methods

PRRS is a globally occurring viral disease in pigs that can lead to foetal death and abortions in sows and to respiratory disease in grower pigs. The economic impact of the disease sparked tremendous global efforts to develop new vaccines, which have to demonstrate to be efficacious against the respiratory infection and associated immune disorders. The impact of PRRSV infection on the immune system is expected to promote and exacerbate secondary bacterial diseases. *S. suis* is a major bacterial pathogen affecting young pigs in the nursery unit with a very high inter- and intra-herd prevalence. Although prevalence is high, the occurrence of disease is limited to more or less frequent disease manifestations in consecutive nursery batches with a disease incidence ranging between 5% and 15%. A concurrent infection with PRRSV has been shown to aggravate and accelerate the course of disease. The high prevalence makes it nearly impossible to find pigs free of S. suis antibodies, and for vaccine studies, where pigs should be naive to the respective pathogen, CDCD pigs are often used as a fall-back solution. However, the drawback of this solution is the use of animals with an unknown impact of the motherless rearing and the lack of a pig specific microbiome in the respiratory tract on vaccine efficacy and disease development. To study these effects and to better characterize this model, we performed an animal study with 6 weeks-old CDCD pigs and conventionally raised pigs (ConR), both from the same farm and the same genetical background (TOPIGS/Norsvin Z70 pigs). Pigs allocation to groups and the used mono- or combined infection protocol is shown in Table 3-1.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014

Table 3-1 Group allocation

Group	n	PRRSV type 1 [D0] 10^5 i.n.	*S. Suis* type 2 strain 10 [D14] 10^7 i.n.
1. CDCD PRRSV/*S.suis*	12	yes	yes
2. CDCD *S.suis*	8	no	yes
3. CDCD control	12	no	no
4. conventional PRRSV/*S. suis*	12	yes	yes
5. conventional *S.suis*	8	no	yes
6. conventional control	12	no	no

The study design and sampling of materials is depicted in Figure 3-1. Pigs of group 1, and group 4 were intranasally infected with PRRSV-1 on study day (D) 0 and followed clinically for two weeks. After two weeks 4 pigs of each of group1, 3, 4 and 6 were euthanized and sampled to study the effects of the PRRSV virus infection on lung immunity and the respiratory microbiome. On day 14, all groups were either infected with a low dose of the virulent S. suis serotype 2 strain 10 (groups 1,2,4,5) or saline (groups 3 and 6) and followed clinically for 6 days. Blood samples (serum and heparin blood) were taken at the times indicated in Figure 3.1. Further blood samples were taken 1 day prior to and 2 days post infection with *S.suis* and preserved in blood RNA tubes. All pigs underwent full necropsy, and tissue samples were taken for bacteriological, pathological and transcriptome analyses. In addition, lung lavage fluid was obtained for immunophenotypic analysis of lung cells by FACS analysis.

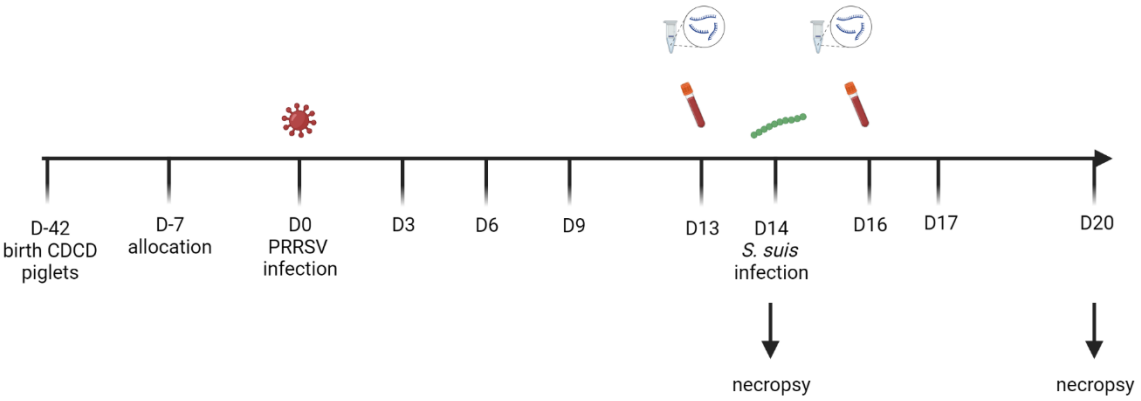

Figure 3-1 Study design

Results

During the viral infection phase part of the study from D0 and D14, a short-lasting increase in body temperature was recorded in PRRSV infected CDCD as well as in ConR pigs and temperature curves were highly similar between both groups, although generally body temperature was consistently about 0.3 to 0.4ºC higher in conventional pigs compared to CDCD pigs. A temperature increase occurred parallel to the starting viraemia at D2/D3 (Fig 3-2). In both groups, no clinical signs (reduced alertness, loss of activity, appetite loss, respiratory

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014

disease signs) were observed; however, 50% of the PRRSV infected CDCD pigs developed discoloration of ears (blue ears) during stress phases between D12 and D14, and CDCD pigs also had a significant lower body weight gain during infection. The discoloration of the skin of the ears is described as a PRRS disease sign, however, the pathogenesis is unclear and most likely related to microvascular changes. The white blood cell count was significantly higher at D0 in ConR pigs ($25*10^6$ cells /ml) than in CDCD pigs ($10*10^6$ cells/ml); PRRSV infection induced a drop of WBC after start of viraemia in ConR pigs, but, interestingly, this was not seen in CDCD pigs.

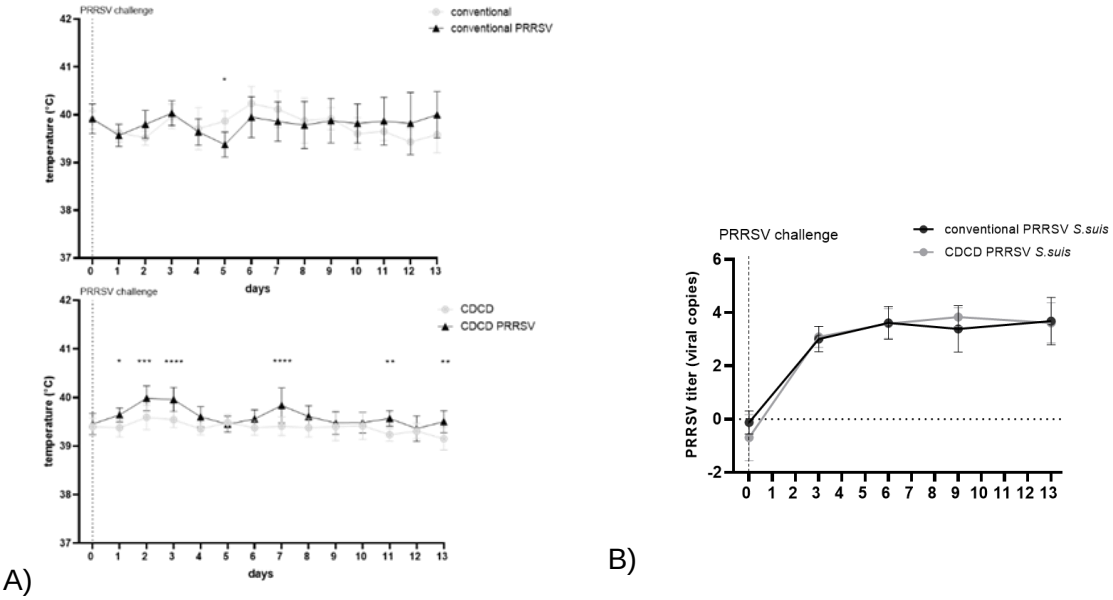

Figure 3-2 A) Body temperature of ConR and CDCD pigs after infection with PRRSV1; B) PRRSV viraemia after infection; viraemia developed in a similar course in both groups and continued over the period of two weeks; the increase in body temperature started with an increasing viraemia, and stayed subfebrile during the virus infection phase.

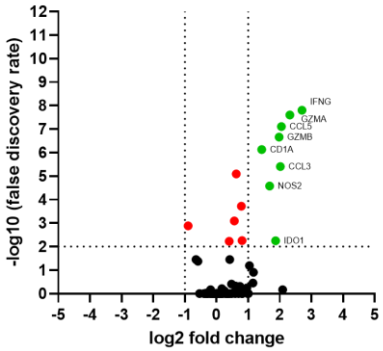

Figure 3-3 Differential host gene expression in blood after infection with PRRSV at D14; NB: a specifically enhanced gene expression profile in response to PRRSV.

At 14 days post infection transcriptome profiles of blood samples were generated by using Nanostring analyses with a focus on acute inflammatory and immune related mRNA transcriptomes. Results showed a more enhanced gene expression profile related to activated monocytes, macrophages, lymphocytes and endothelial cells in CDCD pigs compared to ConR pigs (Fig 3-3).

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014

Necropsies at D14 of 4 pigs of groups 1, 3, 4 and 5 revealed a slightly higher relative lung weight in ConR control pigs than in CDCD control pigs. The PRRSV infections resulted in a significant higher relative lung weight compared to controls in both groups, although no overt clinical signs have been seen (Fig 3-4). Lungs of infected pigs were regularly extended and did not collapse less than in control pigs. Histology confirmed a moderate interstitial pneumonia with dispersed PRRSV antigen in the lung tissue.

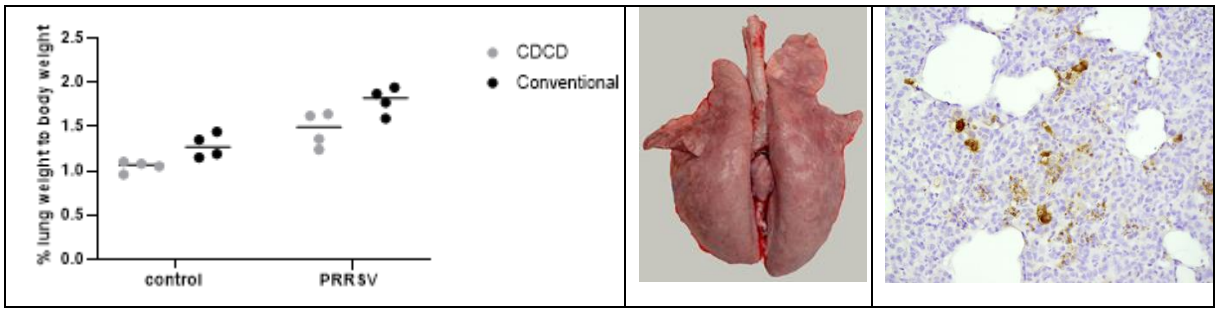

Figure 3-4 Gross pathology results and anti-PRRSV immunohiostochemical staining of lung tissue.

During necropsies, broncho-alveolar lung lavage fluid (BALF) was obtained. In control groups 3 and 6, FACS analyses were performed to distinguish macrophages, neutrophils and various lymphocyte populations. Non-infected ConR pigs (group 6) had nearly twice as much cells with a significantly higher number ($2.9*10^8$) of cells in the BALF than in CDCD pigs ($1,5*10^8$). The BALF cell composition is dominated in control pigs by macrophages, ranging between 85% of cells in ConR and 90% of cells in CDCD pigs. The proportion of lymphocytes was slightly higher in ConR pigs than in CDCD pigs (ca. 10%). CD8+ cells were in a significantly higher proportion of CD3+ cells represented in the control ConR pigs than in the control CDCD pigs (Fig 3-5). Macrophages are target cells of PRRSV and the PRRSV infection reduced the proportion of macrophages in both groups to less than 10%.

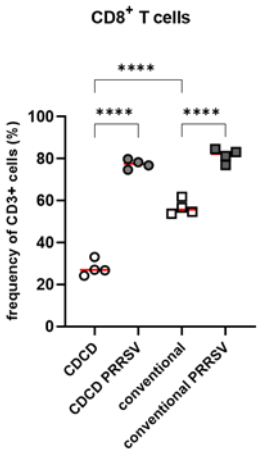

Figure 3-5 Proportion of CD8+ T-cels in broncho-alveolar lung lavage of control and PRRSV infected pigs.

At D14 after infection, the total number of cells in BALF was similar to the control pigs in both groups, however, a significantly higher proportion of neutrophils occurred in CDCD pigs than

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014

in ConR pigs, whereas in both rearing groups the proportion of CD8 of CD3 positive cells was highest with ca. 80%, but not different between groups. Apparently, inflammatory cell migration into the alveoli after virus infection is altered in the lung tissue of CDCD pigs compared to ConR pigs.

Experimental infections with *S. suis* regularly lead to typical disease and lesions in approx. 80% of the pigs. Therefore, in this study the infection with *S. suis* was performed with a low infection dose. The single *S. suis* infection in group 4 revealed, that ConR pigs indeed did not develop clinical signs nor increase in body temperature and pigs seem to be able to control the infection. In contrast, all of the CDCD pigs (group 2) developed fever and typical signs of disease, and during necropsy the typical inflammation of joints, serosae of brains were observed and findings were confirmed by bacteriology results, where *S. suis* was cultured in all pigs from abdominal and pleural serosal surfaces, joints and meninges. Although *S. suis* is not an obligatory lung pathogen, aerosol administration to reach the upper airways and tonsils is a common administration route in infection studies and considered to reflect a natural infection. Selected mRNA expression profiles (by Nanostring™ analysis) on blood samples from 2 days after infection remain to be analysed. The results of the bacterial mono-infection demonstrate the higher susceptibility to disease development in CDCD pigs, where the lack of colostrum uptake and immune development and differences of bio-environment may influence the efficiency of the innate immune response.

Infectious diseases are regularly initiated by a specific pathogen and can be aggravated by co-infections, which is a quite common sequela of respiratory tract infections, best known as viral/bacterial co-infections. Due to the relevance of co-infections and polymicrobial diseases, the mechanisms of disease initiation and aggravation by co-infections have gained more interest in recent years, especially in pigs. The impact of the used animal model in regard to peri-natal rearing and exposure to a specific bio-environment has been addressed in this study in groups 1 and 4.

PRRSV infection increased the development of S. suis disease in CDCD pigs, but also in ConR pigs, with the difference, that disease progression was slightly faster in CDCD pigs and humane endpoints had to be applied in CDCD pigs earlier than in ConR pigs (Fig. 3-6).

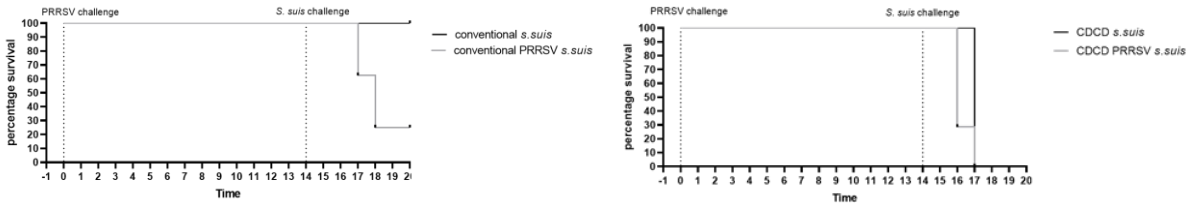

Figure 3-6 Survival rate of pigs after single infection with S. suis and after primo-infection with PRRSV followed by an infection with S. suis 14 days later; left panel: ConR, right panel CDCD pigs.

Body temperature after *S. suis* infection was in both CDCD groups (single or co-infection) significantly higher (and febrile) than in the ConR group, but fever occurred also in the PRRSV

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014

pre-infected ConR group. Strikingly, bacteraemia measured at two days post infection was detected in only two pigs of the PRRSV pre-infected ConR group, whereas the majority of the CDCD pigs showed translocation of bacteria into the blood (Fig. 3-7). The circulating WBC were significantly lower in the CDCD groups, but as a consequence of the bacterial infection, WBC counts in CDCD pigs raised to similar levels as in ConR pigs. In how far this tremendous acquisition of leucocytes also impacts the competence of these cells to fight the bacteraemia will be examined by further immunological assays and transcriptome analyses.

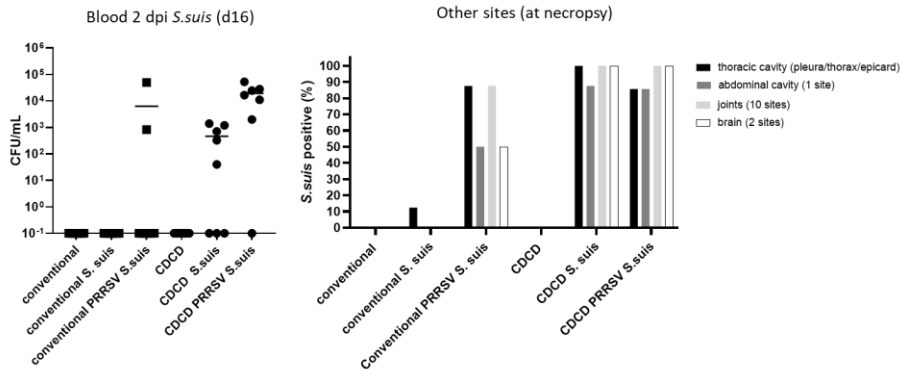

Figure 3-7 Occurrence of bacteraemia at 2 days post infection and isolation of S. suis in various target tissues at necropsy.

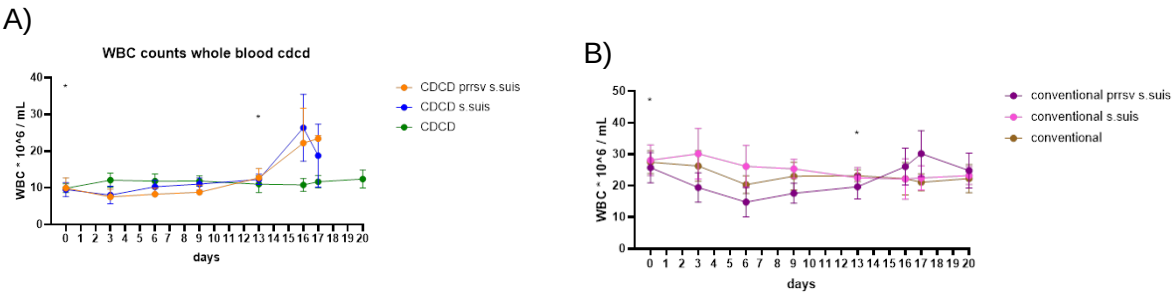

Figure 3-8 White blood cell count after PRRSV and S. suis infection in CDCD (panel A) and ConR (panel B) pigs.

3.3 Avian influenza virus infection in chickens, Pekin ducks and mallard ducks (FLI)

Chickens, Pekin ducks and mallard ducks were used in virus characterization and transmission studies, backed by comprehensive in-vitro approaches (establishment of in-parallel workflow for AIV characterization in embryonated duck and chicken eggs and cell culture models using also primary bird-embryo cells).

In the past 25 years, one highly pathogenic H5 lineage (A/goose/Guangdong/1/1996(H5N1)) and its sub-lineages and reassortants have become dominant. With continuous increases in total case numbers, range of affected bird and mammalian species, outbreaks in poultry holdings, mass cullings and deceased animals, gs/GD H5 has reached inconceivable magnitude. Clade 2.3.4.4 B H5N8 2016 and H5N8 2020 reassortants were characterized in these models. Particularly the duck model has turned out to be of high value due to its natural resistance against AIV (presumably RIG-I pathogen recognition receptor dependent), reservoir traits and transmission vector. With this approach AIV pathogenesis was mirrored in two major

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014

players (chickens and ducks), showing that chickens are highly sensitive to fatal disease but also ducks, which were believed to show at least partial resistance, although progression and clinics were differing from those in chickens (neurological manifestation, slower progression). Next, the role of mallard ducks in 2.3.4.4 B spreading dynamics was evaluated. Housing mallards as sentinels for wild-bird AIVs under semi-natural conditions, allowing contact to wild bird populations, enables for induction of naturally acquired immunity against enzootic AIVs. These immunologically primed ducks may now be used in infection studies in a high containment facility, which enables precise studies of high-impact AIVs. Despite bearing the same genetic background, Pekin ducks (domesticated) and the sentinel-mallards (non-domesticated) showed differing susceptibility towards 2.3.4.4 B AIVs, which can be traced back rather to pre-existing immunity than triggering of RIG-I or related antiviral mechanisms and pathways in waterfowl. This also explains H5 gs/GD dynamics in wild bird populations and the varying degrees of severity over the years: depending on pre-immunity in populations, spreading dynamics are accelerated. Despite of 2.3.4.4 B infection, clinically healthy animals, as mirrored by our studies, are the most important vector for transcontinental AIV spread (Asia-Europe-Americas).

Besides gs/GD H5 also avian H7 influenza viruses have emerged. Among these high-consequence viruses are Asian H7N9 (low and highly pathogenic) reassortants. These comprise an increased zoonotic potential, which has resulted in several waves of severe and fatal human H7N9 cases in China and Southeast Asia in the past decade. Due to the high potential of H7N9 Asia to cause zoonotic disease and deaths and a mainly H7N9-naive bird population, they are strictly handled under highest safety conditions in BSL-3 facilities. Vector mediated spread of H7N9 Asia with wild waterfowl species from Asia to Europe has not been reported, but would be of dramatic consequence. Hence H7N9 adaptation to Pekin ducks, which again serve as a model for numerous waterfowl species, pathogenesis and transmission efficiency (shedding kinetics) was evaluated. Besides, H7N9 Asia was also characterized in chickens in a parallel approach. Disease severity and shedding dynamics resembled those known for H5 highly-pathogenic viruses. However, marked differences in waterfowl adaptation of the three isolates, which were tested, could be shown.

Results have been published (Koethe et al., 2020, Emerg Microbes Infect, https://doi.org/10.1080/22221751.2020.1713706) and two further publications are still pending. The animal models and especially the experimental approaches, which were implemented with this work, were also adopted by others (e.g. Ahrens et al., 2022, Emerg Microbes Infect), especially relying on experiences with model-species as mallards and animal-welfare oriented housing conditions, which increase validity of a study, since side-effects due to the impact of improper holdings, can be excluded.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014

Ultimately, bundling competences in handling high-consequence pathogens at certain sites allows national and international partners to participate and boosts the response and reaction efficiency without the need of implementing multi-site parallel structures. Target-oriented improvement of interventional measures for disease prevention and control can easily be realised with this approach and ultimately helps to improve preparedness towards emerging (zoonotic) pathogens.

3.4 Avian influenza virus infection and transmission in turkeys and quail (PIWET)

An avian influenza (AI) transmission experiment was carried out in 3-week-old turkeys divided into 5 groups (I-V), 5 birds in each group. At day 0, turkeys of the group I were infected intranasally and intraocularly with 10^6EID_{50} of a turkey-adapted low pathogenicity AI virus (LPAIV) belonging to the H7N7 subtype and observed for 5 days. At day 1, turkeys of the group II were added to an adjacent cage with free airflow (indirect contact transmission) for the 8-day period. Similarly, birds of the groups III-V were put in the cages placed next to the cage with birds from the previous group. Oropharyngeal and cloacal swabs were collected at 3 and 5 days post-infection (dpi, all groups) and additionally at 8 dpi (groups II-V). The swabs were tested by RT-qPCR. All birds remained healthy without obvious clinical signs. AIV-RNA was detected in turkeys of group I and II at every examined time-point in all tested swabs. In turkeys of group III, only an oropharyngeal swab from one bird was positive at 3, 5 and 8 dpi. All samples from turkeys of groups IV and V were negative. The results suggest that even poultry-adapted LPAIV have limited capacity of sustained transmission in highly susceptible species (turkeys) through indirect contact.

Two more rounds of experiments were performed in which the H7N7 subtype LPAIV (isolated from domestic turkeys) was passaged through 3-week-old turkeys (2nd round), and alternately between 10-week-old quails and turkeys (3rd round). Prior to experimental inoculation, swabs and serum samples were collected and tested by RT-qPCR and ELISA to exclude any previous or ongoing AIV infection. In the 2nd experimental round 25 birds were used, while in the 3rd round necessitated the use of 50 birds: 25 quails and 25 turkeys. In each round, the birds were randomly divided into 5 groups (I-V), five birds in each group. The birds from group I of each experimental round were inoculated via the oculo-nasal route with a dose of 10^6EID_{50} of LPAIV H7N7 in a 100µl volume per bird. At 1 dpi, five contact birds from group II were introduced to the directly adjacent cage. At 5 dpi, birds from group I were euthanized and birds from group III were placed in another adjacent cage. Birds from groups IV and V were placed in cages right next to the birds representing the previous passage. The experiment was designed so as to enable indirect contact between respective groups of birds over the 4-day period whereas the whole observation period of each group (except group I) was 8 days. The 3rd round of the experiment differed in that quails and turkeys were used alternately. At 3, 5 dpi (all groups)

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014

and 7 dpi (groups II-V) oropharyngeal and cloacal swabs were collected and tested by RT-qPCR. No clinical signs or mortality were noted in quails and turkeys from all groups or experimental rounds. In the 2nd experimental round, all turkeys from I and II group showed oral and cloacal shedding at 3 and 5 dpi. Additionally, one bird from group III showed the presence of viral RNA also at 5 and 7 dpi but only in oropharyngeal swabs. AIV-RNA was not found in turkeys of group IV and V and in quails of III, IV and V group. In the 3rd round of the study, the virus was detected in all quails of group I at 3 dpi and 5 dpi mainly in the oropharyngeal swabs. No viral RNA was found in turkeys from groups II onwards and therefore the decision to terminate the study was made.

4 Conclusions

With the work performed by EMC, a significant technical improvement has been achieved, by which it gets possible to better quantify the presence of virus particles in air streams. Viral doses matter, and the technology offers the opportunity to better monitor and design airborne transmission studies and provide better information about relevant infectious doses. Work performed by EMC in response to the COVID-19 pandemic evidenced the transmission of SARS-CoV-2 in experimentally infected ferrets via contact and via the air.

The comparative work in CDCD piglets and conventionally reared piglets performed by WBVR demonstrated the influence of rearing conditions, like motherless rearing, lack of colostrum and exposure to “pig specific” microbial ambient air and bio-environment, which result in a higher susceptibility to disease development in CDCD pigs after single bacterial infection and viral/bacterial co-infection. Differences in systemic and local immune parameters between the two rearing condition have been shown on the cell and mRNA expression level before and after infection(s) and might explain differences in the outcome of studies with variable animal parameters. The results confirm that the choice of the used animal model influences the outcome of studies and that comparison between infection studies even when using the same animal species need sufficient in-depth knowledge about the impact of housing and rearing on the host immune response and physiology.

The works performed by FLI and PIWET evidenced marked differences in the susceptibility to AIV infection or virus transmission efficiency among the various domestic poultry species studied. This confirms the requirement to study avian influenza in a variety of avian host and the need for reliable and proven animal infection models.

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement N°731014