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Coronavirus: Infection of Piglets and Men

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Coronaviruses were first described in early sixties, when samples from poultry, pigs and/or calves with clinical signs of a mild respiratory disease (and/or showing diarrhoe of unknown etiology) were inculated into celll cultures and later on, if revealing cytopathic changes, examined by electron microscope). The term Coronavirus was used to describe the novel enveloped virus particles with typical spikes (about 20 nm long) present in their outer membrane. The pig Coronavirus called porcine respiratory and reproduction syndrome virus (PRRSV) has been classified as member of family Arteriviridae (order Nidovirales), along with the equine arteriitis virus and/or the lactate dehydrogenase elevating virus of mice [1]. The virions form small, enveloped particles (50-65 nm in diameter) harboring a relatively long (approximately 15 kb in size) single strand RNA genome [2]. The term Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) has been recently introduced in association with a novel Coronavirus (nCoV) which has emerged in China (in the city of Wuhan, Hubei province), from where it quickly spread all over the world. The nCoV has also been called Coronavirus 2019 (CoV-19) by some investigators, while others termed it Coronavirus 2 (CoV-2) [3]. Within a relatively short period of 6 months (from March to August 2020), a novel Coronavirus (formerly known as the 2019 coronavirus [2019-CoV]) again originating from China (namely from the city of Wuhan in December 2019), caused the emergence of a novel coronavirus disease 2019 (COVID-19). The new CoV has been called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [4]. The reported outbreak occurred in the Hubei province and had a possible common exposure link to the Wuhan's South China Seafood City (ECDC) market. At the very beginning of the 2019-nCoV outbreak, many details remained unclear, except for the fact that the virus was transmitted by direct exposure [5]. Namely, the Wuhan fish and wild animal market sold live animals such as poultry, bats, marmots and snakes, which might have been the source of infection [6]. Even though the majority of COVID-19 cases was reported from continental China, the island of Taiwan has been found involved as well [7]. Unlike to previously (the Guandong province in the year 2002 and/or 2003) described "classical" coronavirus (CoV), the novel one quickly spread all over the world infecting nearly 30 million people (at least 3-4 %), while some had signs of severe lung disease. Therefore, WHO has declared the recent outbreak for public health emergency of international concern [8].

The porcine respiratory and reproduction syndrome virus (PRRSV) forms small, enveloped particles (50-65 nm in diameter) harboring a relatively long (approximately 15 kb in size) single strand RNA genome [9]. The viral RNA (vRNA) is a positive-sense molecule with terminal cap at 5′-end and a poly-A repeat at 3′-end [10]. In the course of virus replication, the vRNA is copied as whole, when synthesized via a full length negative-strand RNA intermediate. The vRNA sequence begins with 2 (two) long open reading frames (called ORF1a and ORF1b), which together comprise about 75% of the total genome). [11]. This portion of the genome specifies 14 non-structural proteins (nsps) which are formed by cleavage of the both translated polyproteins. Of special importance are, for example, two non-structural proteins (nsp9 and nsp12), which function as vRNA replicase, also termed RNA-dependent RNA polymerase (RdRp) [12]. The rest of the genome encodes 7 structural proteins, out of which 5 are glycoproteins (designated GP2a/Gp2, GP2b/E, GP3, GP4 and GP5) along with the M (membrane) protein and the N nucleoprotein [13].

Regarding to the structure of the vRNA, the PRRSV has been classified as a member of family Arteriviridae (order Nidovirales), along with the equine arteriitis virus and the lactate dehydrogenase elevating virus of mice [14]. In the course of vRNA replication, a total length (genomic) minus strand is generated, which serves as template for the synthesis of new vRNA molecules. During viral mRNA synthesis, the negative sense RNA sequence is being formed first; then a set of positive sense nested subgenomic (sg) RNA molecules is transcribed. Finally, the full set of minus sense subgenomic (sg) RNAs is formed, which becomes a template for the synthesis of functional positive sense sg mRNAs [15]. Both strands are complementary to each other; their coterminal 3′-ends are equipped with a common leader sequence at their 5′-ends [16, 17]. The viral genome reveals several (but at least two) conserved transcription regulatory sequences (TRS), which are located either in the front of ORF1a (encoding the structural protein GP2a) or before ORF2a (encoding the envelope glycoprotein Gp2b/E).

The classical PRRSV strains which were isolated in the US (VR2332) and/or in Europe (Lelystadt) differ at both, by serological as well as genome examinations [18]. Experimental infection with the PRRSV isolates can be lethal in newborn and/or 3-week-old piglets. A key event of the infection process is the involvement of porcine alveolar macrophages, which are the most important virus target also mediating virus spread [19]. To date, at least two macrophage surface molecules are known as entry mediators: the siglec sialoadhesin and a scavenger receptor CD163 [20]. The PRRSV induced pneumonia is characterized

by thickening of inter-alveolar septa due to infiltration with macrophages and by the presence of occasional inflammation and cell debris within the alveoli itself [21]. Also alveolar pneumocytes of type II may be found PRRSV antigen positive along with the hyperplasia of peribronchial lymphatic tissue [22]. The severity of lung lesions may vary from relatively mild to quite extensive. The viral genotypes can differ in their pathogenicity, namely the Type 2 North American PRRSV induces more severe respiratory disease than type 1 European virus. Nevertheless, mild thickening of interalveolar septi can be mistaken with focal thickening of inter-alveolar septa in combination with slight infiltration of peri-bronchial connective tissue (referred to as mild non-specific interstitial infiltrate, MNSII), was occasionally seen in a proportion of non-infected control piglets and interpreted as unrelated to PRRSV infection [23]. In this paper we describe the correlation of the lung lesions as seen at histological examination slides stained with HE on comparision to the immunohistochemical detection of viral N-protein along with the results of serological tests for N-protein antibodies.

The viral RNA (vRNA) is a positive-sense molecule with terminal cap at 5´-end and a poly-A repeat at 3´-end [2]. It encodes 2 (two) long open reading frames (ORF1a and ORF1b), which comprise nearly 75% of the total genome sequence. The latter part of the genome specifies 14 non-structural proteins (nsp) formed by cleavage of the 2 corresponding translated polyproteins. Of special importance are the non-structural proteins 9 (nsp9) and 12 (nsp12), representing the vRNA replicase, termed also RNA-dependent RNA polymerase (RdRp). The rest of the vRNA sequence encodes 7 structural proteins, out of which 5 are surface glycoproteins (designated GP2a/Gp2, GP2b/E, GP3, GP4 and GP5) in contrast to the M (membrane) protein and the internal virion nucleoprotein N [24]. The viral genome also reveals (at least two) conserved transcription regulatory sequences (TRS), located in the front of the structural protein GP2a gene and the envelope glycopreotein (Gp2b/E) gene. In the course of virus replication, the vRNA is copied via a full length negative-strand intermediate. Thus, first a complete negative sense RNA sequence is generated, which serves as template for the synthesis of the new (genomic) vRNA. For viral mRNA synthesis, a set of positive sense nested subgenomic (sg) RNAs is transcribed. Briefly, the minus sense subgenomic (sg) RNAs is used as template for the synthesis of functional positive sense sg mRNAs [25]. Both strands are complementary to each other and at their coterminal 3´-end and are equipped with a common leader sequence at their 5´-ends [26]. The vRNA has of 2 long open reading frames (ORF1a and ORF1b), which comprise about 75% of the total genome sequence. This portion of the genome specifies 14 non-structural proteins (nsp) formed by cleavage of the 2 corresponding translated polyproteins. Of special importance are the non-structural proteins 9 (nsp9) and 12 (nsp12), representing the vRNA replicase, termed also RNA-dependent RNA polymerase (RdRp). The rest of the genome encodes 7 structural proteins, out of which 5 are glycoproteins (designated GP2a/Gp2, GP2b/E, GP3, GP4 and GP5) in addition to the M (membrane) protein and the N nucleoprotein [4]. The viral genome has (but at least two) conserved transcription regulatory sequences (TRS), located in the front of ORF1a and ORF2a, which encode the structural protein GP2a and the envelope glycopreotein (Gp2b/E).

The standard hygiene measures seemed not being enough to prevent the quick spread and worldwide transmission of the virus, in the beginning they were implemented aiming to 1) slow the spread of illness; 2) provide time to better prepare in any state and its local health department, the health care systems, businesses, educational organizations, and the general public in an event that the expected widespread transmission really occurs; and in order to 3) characterize COVID-19 in the public health recommendation guides [27]. The development and deployment of medical countermeasures, including precise diagnostics, therapeutic recommendation, and not excluding the efforts aiming for vaccine development [28]. Asymptomatic cases were diagnosed based on positive viral nucleic acid test results, but without any COVID-19 symptoms, gastrointestinal, or respiratory symptoms, and no significant abnormalities on chest radiograph [29]. In addition, the transmission of COVID-19 through asymptomatic carriers via person-to-person contact was observed in some reports [30,31]. Using a large sample for estimation, suggested that the median incubation period was only 3.0 days, but could be as long as 24 days. The mean incubation period, was 5.2 days ranging from 2.1 to 11.1 days.

Patients with ARD defined as laboratory-confirmed COVID-19 cases had respiratory symptoms; however, chest computed tomography (CT) did not reveal signs of pneumonia [32]. Patients with pneumonia defined as COVID-19 cases had both respiratory symptoms and pneumonia on chest radiograph [33]. The latter category includes severe pneumonia of either respiratory rate over 30/minute, SpO2 93%, or PaO2/FiO2 over 300 mmHg) and a critical condition, characterized by respiratory failure requiring mechanical ventilation, shock, or other organ failure requiring ICU management [34]. Patients with pneumonia are older, with a higher prevalence of smoking history, more underlying diseases, and were more likely to have fever, myalgia/fatigue, dyspnea, headache, and nausea/vomiting compared to patients with ARD (all differences are at $p < 0.05$). In addition, pneumonia cases presented a higher white blood cell count and neutrophil count, but had a reduced leukocyte count compared to ARD cases [35]. These patients received more antibiotics and antiviral therapy and were more likely to require oxygenation therapy, mechanical ventilator, renal replacement, and extracorporeal membrane oxygenation [36].

Person-to-person transmission of 2019-nCoV has been confirmed and asymptomatic individuals have been identified as potential sources of infection [37-40]. The identification of cases and contacts of persons with COVID-19 and the recommended assessment, monitoring, and care of travelers arriving from areas with substantial COVID-19 transmission were similar than with the previous influenza virus pandemic [41]. The differences in infectivity of coronaviruses can be attributed to the differences in the rigidity of their shells which can be evaluated using computational tools for predicting intrinsic disorder predisposition of the corresponding viral proteins [42].

The estimated reproductive number of 0.3 was obtained from a small number of infected persons with imperfect information in the very early stages of the outbreak [43]. therefore the reproductive number of 2019-nCoV is likely to be similar to that of the 2002/2003 severe acute respiratory syndrome (SARS) coronavirus during the pre-intervention period (range, 2 to 3) and that of the 2009 pandemic A/H1N1 influenza virus in the United States (range, 1.3 to 1.7) [44, 45]. Owing to these observations, the current control measures for 2019-nCoV, including the quarantine and an observation period of 14 days for suspected cases, can be considered for appropriate.

The structural proteins (and/or glycoproteins) by any Beta-coronavirus (B-CoV) strain are encoded by four regularly present structural genes, namely the spike glycoprotein (S, former E2), the envelope glycoprotein (E, former sM), the membrane glycoprotein

(M, former E1) and the nucleocapsid protein (N) [46]. The 27.2 kb long single stranded negative sense viral RNA (vRNA) sequence has an untranslated region at its 5´-end (5´- UTR) along with a short leader sequence (LS) which continues into the two relatively long open reading frames (ORF 1a/b) encoding corresponding polyproteins (plp1a and plp1b). These become cleaved by an endogenous peptidase to form at least 10 non-structural viral polypeptides (nsp) involved in vRNA replication. The next four genes encode the above mentioned structural proteins; they are interrupted by sequences specifying the so called accessory proteins (in the case of CoV-2 these are ORF 3, 6, 7a, 7b, 8 and 9b). The latter are located either in between S and E sequences (ORF 3) or between M and N (ORF 9b is an exception, since it is positioned directly in the N sequence). Finally, the sequence of CoV RNA ends by a short untranslated region forming the 3´-UTR sequence [47-49]. When comparing CoV-2 with the earlier CoV isolates, the key variation was found in the ORF 3 sequence region. Taken together, the CoV genome might show the following sequence:

5´-LS--p65/Plp1a/ORF1a/NSP1--Plp1b/ORF1b/NSP2 (a poliovirus type protease)—NSP3 --NSP4—NSP5--NSP6--NSP7--NSP8--NSP9 (the RNA polymerase)--NSP10 (helicase/NTPase) --NSP11--NSP12--NSP13--S-E-M-N-3´

The life cycle of SARS-CoV-2 in the susceptible host cells begins by binding of the S protein to a corresponding cellular receptor, namely that for acetylcholinesterase (ACE2). After receptor binding, a conformation change within S protein facilitates the fusion of the virion membrane with the cell membrane, which activates a transportation pathway along the cellular endosomal reticulum (ER). The virus coded polymerase produces a series of sub-genomic mRNAs transcribed from the released vRNA by a process called discontinuous transcription. In the region of cellular ER and Golgi apparatus the set of newly formed transcripts is finally translated into relevant viral proteins. These along with the transcribed novel vRNA are subsequently assembled into new virions, which are via the cytoplasmic vesicles transported back to cell membrane in order to get released out of the cell.

The PRR syndrome in piglets is characterized by high mortality, reproductive failure (late-term abortions and stillbirths, premature farrowing, mummified pigs in pregnant sows) and a severe respiratory disease (interstitial pneumonia). The disease occuring in the nursery and among growing/finishing piglets causes significant economic losses to the swine industry worldwide. The corresponding virus (PRRSV) replicates mainly in the porcine alveolar macrophages (PAMs) and dendritic cells (DCs). The virus also causes persistent infection eliciting antibody dependent enhancement (ADE) and ocassional immunosuppression. Being a member of the family Arteriviridae, it belongs to the order Nidovirales together with the Coronaviridae and Roniviridae families [50]. PRRSV was originally divided into European type 1 and North American type 2 genotypes. Later on, the East European PRRSV isolates have been found to be of the European genotype, but forming different subtypes. A novel virus, namely the Belarusian strain Lena, has been recently characterized as a highly pathogenic East European subtype 3, which differs from European subtype 1 Lelystad and North American US5 strains at genetic as well as antigenic levels.

Numerous results suggest that PRRSV may utilize multiple strategies of spread in the infected pigs, including subversion of the host innate immune response, inducing an anti-apoptotic and anti-inflammatory state as well as developing ADE.

The PRRSV induced immunosuppression might mediate apoptosis of infected cells, which causes depletion of immune cells and induces an anti-inflammatory cytokine response due to which the host is unable to eradicate the primary infection. The initial antibodies do not confer protection and can even be harmful by mediating an antibody-dependent enhancement (ADE), since they can facilitate the virus entry of into targets cells in vitro. To characterize the humoral immune response direct enzyme-linked immunosorbent assays (ELISA) can be used including different mainly recombinant PRRSV antigens. For example, the kinetics of antibody responses directed against nonstructural virus coded proteins (nsp) can be analysed in pigs experimentally exposed to the virus [51]. In such case, high antibody reactivities especially against nsp1, nsp2, and nsp7 were noted. Among the latter, nsp7 recombinant protein based ELISA showed good sensitivity and specificity most suitable for diagnostic development especially for identification and differentiation of type 1 and type 2 PRRSV. Several non-structural proteins (such as nsp1, nsp2, nsp5, nsp7 nsp9, nsp10 and nsp11) have been implicated in the induction of IFN-γ and also in the development of the cell-mediated immune response. On other hand, the induction of neutralizing antibodies (NAs) may be delayed and/or their levels may remain low, which is not only the problem of early diagnostic, but is also of importance regarding effective virus elimination. NAs may protect against disease if present in sufficient quantities before infection, but they do not seem to be essential for clearing virus in blood during the course of the infection. PRRSV is able to modulate innate responses, probably through the regulation of IFN-α and IL-10 responses [52]. In general, fever occurs with a probability ranging from 67% to 98%, cough by at least from 43% but up to 81%; the shortness of breath may be present in 31% to 55% of CoVID-19 cases and finally, the frequency of myalgias remains as low as 3% to 11%, but never exceeding 44%. Patients with pneumonia were older, with a higher prevalence of smoking history and more underlying diseases. They were more likely to have fever, myalgia/fatigue, dyspnea, headache, and nausea/vomiting as compared to patients with a simple ARD, revealing a statistical difference of $p < 0.05$. In addition, the pneumonia cases have presented higher white blood cell and neutrophil counts, while the simple ARD cases had rather a reduced leukocyte count [53]. The pneumonia patients, as a rule, received more antibiotics and/or antiviral therapy and later on they were more likely to require oxygenation therapy, mechanical ventilator, extracorporeal membrane oxygenation and even renal replacement [54].

As described, PRRSV replicates predominantly in the lung alveolar macrophages, can induce prolonged viremia, and cause persistent infections lasting for months after initial infection. PRRSV strongly modulates the host's immune response and changes its gene expression. Studies showed that PRRSV inhibits type I interferons (IFN-□). Regarding cell-mediated responses, development of PRRSV-specific gamma interferon-secreting cells (IFNgamma-SC) and interleukin 4-secreting cells (IL4-SC) in PBMC was examined by ELISPOT assay. Using this technic, no IFNgamma-SC was detected until day 14 p.i., whereas for IL4-SC, such differences were not seen. Concurrently with the onset of viremia and the development of clinical signs, serum haptoglobin levels and interleukin 10 (IL10) in PRRSV-stimulated PBMC-culture supernatants increased significantly. These results are compatible with the model of pathogenesis in which the immune response does not fully control the outcome of infection.

The PRRSV replication and its spread in the body subverts the host innate immune response as well when highjacking its lipid

metabolism and inducing an anti-apoptotic and anti-inflammatory state. The latter is indicated by suppressing the expression of serine proteinase inhibitor 2 (SPI 2), IFN-□, and down-regulation of the expression of pro-apoptotic genes such as B-cell lymphoma 2 (BCL-2) antagonist/killer (BAK) and the BCL-2 associated X (BAX). Whereas BAX resides predominantly in the cytosol, BAK is constitutively localized to the outer mitochondrial membrane; both form toxic mitochondrial pores in response to cellular stress. Furthermore, the APR-1, i.e. the Adenomatous polyposis coli (APC) protein which is a Wnt signaling component along with a microtubule-associated protein SARP3 (several ankyrin repeat protein 3), may be down-regulated. Both were shown to interact with all isoforms of PP1 (protein phosphatase 1). Infections of N-PRRSV viruses resulted in fever and inflammatory response, as indicated by high expression of proinflammatory cytokines and chemokines, adhesion molecules, inflammatory enzymes and their receptors, such as IL-1□, IL8, SELL, ICAM, CCL2, CXCL9, CXCL10, B2M, proteasomes and cathepsins. This was compounded by cell death and elevated expression of NFKBIA, XAF1, GADD45A, perforin, granzymes, and cytochrome C, coupled with increased ROS-mediated oxidative stress, as indicated by up-regulated expression of cytochrome b245. Taken together, the N-PRRSV infection may have resulted in an excessive immune and inflammatory response that contributed to tissue damage [55].

There is no clinically approved antiviral drug or vaccine available to be used against COVID-19. As of now, there is no specific antiviral medication available for COVID-19 treatment, and also no vaccine is currently available. Health care providers generally treat the symptoms by using oxygen therapy for patients with severe infection. However, few broad-spectrum antiviral drugs have been evaluated. A potential antiviral treatment of human CoV has been recommended using drugs such as Lopinavir/Ritonavir (400 mg/100 mg), nucleoside analogues, neuraminidase inhibitors, Remdesivir, the peptide EK1, arbidol, RNA synthesis inhibitors (such as TDF, 3TC) and/or certain anti-inflammatory drugs including IFN-alpha (5 million Units/dose). IFN-alpha is a broad spectrum drug, which can be used, for example, to treat hepatitis B [56]. Lopinavir is a protease inhibitor showing anti-CoV activity in vitro. It has been used to treat infection by human immune deficiency virus, together with ritonavir as a booster. For SARS treatment, there was found that in contrast to ribavirin alone, patients treated with lopinavir/ritonavir as well as ribavirin had a lower risk of the so called acute respiratory distress syndrome (ARDS) and/or death. Nevertheless, as shown in mouse experiments using the Middle East Respiratory Syndrome (MERS)-CoV, Remdesivir may have the best CoV treatment potential. Namely, it can effectively reduce the virus titer in infected mice, it even improves the lung tissue damage. Its effect may be better than that of the treatment using Lopinavir/Ritonavir combined with interferon [56].

China has relied on the use of the anti-viral drug Favilavir to treat the symptoms of COVID-19. This medication was initially developed by Toyama Chemical to treat nose and throat infections. Although the results of the study have not yet been published, it has been assumed that the drug has proven effective (at least in part) in treating symptoms of COVID-19 in a clinical trial of more than 70 patients with minimal side effects. Favilavir is an another new antiviral drug that was approved in Japan in 2014 to treat influenza, but currently also has for treating COVID-19, but not by the U.S. Food and Drug Administration (FDA). Remdesivir (GS-5734) is a broad-based antiviral drug originally designed to target Ebola and was developed by Gilead Sciences. It inhibits viral replication through premature termination of RNA transcription, which disrupts the virus's ability to reproduce. China announced that clinical trials of Remdesivir, have officially started in Wuhan to test its efficacy against COVID-19. Moreover, one clinical trial has also been approved by the FDA in the United States. However, the efficacy and safety of Remdesivir in patients still need to be further clinical studies.

Chloroquine and Hydroxychloroquine are drugs used to treat malaria, as well as chemoprophylaxis; and certain inflammatory conditions to include rheumatoid arthritis, lupus and the blood disorder porphyria cutanea tarda, respectively. They have been approved by the FDA to be tested against COVID-19. Researchers have found that both drugs have in vitro activity against SARS-CoV and SARS-CoV-2, with hydroxychloroquine having relatively higher potency against SARS-CoV-2. Based on these results, chloroquine and 5-hydroxychloroquine are currently recommended for treatment of hospitalized COVID-19 patients in several countries, including in the U S. A Chinese study showed that when chloroquine was tested on more than 100 patients, it had superior results compared to a control drug inhibiting the exacerbation of pneumonia, improving lung-imaging findings, promoting a virus negative conversion and shortening the disease course. However, both Chloroquine as well as Hydroxychloroquine may cause frequent side effects, such as worsening vision, nausea, digestive disorders and more severe cases can lead to heart failure. A man in Arizona died and his wife was in critical condition after taking chloroquine prophylactically to prevent SARS-CoV-2 infection.

Lopinavir/Ritonavir are sold under the name Kaletra by AbbVie and are designed to treat HIV (AIDS). To evaluate the efficacy of lopinavir/ritonavir for SARS-CoV-2 infection, 99 patients with positive infections were treated with lopinavir/ritonavir. No benefit was observed with lopinavir/ritonavir treatment compare to standard care. However, in South Korea, a 54-year-old man was given a combination of these two medications and had a significant and substantial decrease in the levels of the β-coronavirus. According to the WHO, there may be benefits to using lopinavir/ritonavir with other drugs such as interferon-β, oseltamivir or ribavirin.

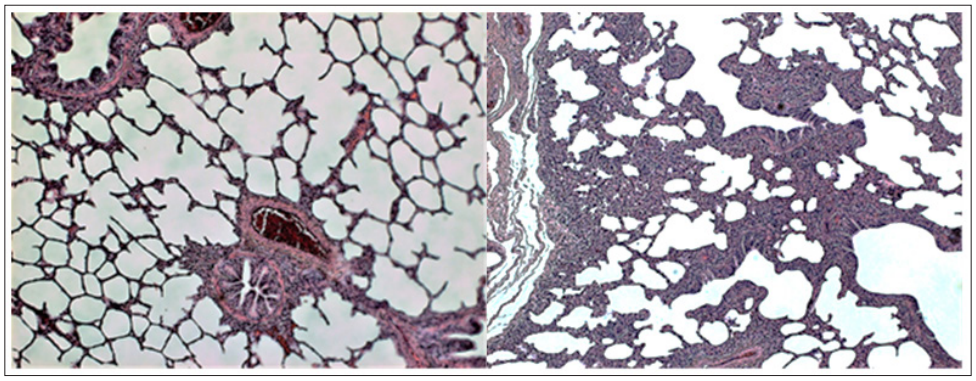

Figure 1: Histological picture of the lung tissue in uninfected (control) piglets.

A. In the left (piglet no. 5). The normal lung structure at low power view shows thin interalveolar septa devoid of any infiltrate; in the peribronchial (and/or perivascular) connective tissue a few mononuclear cells (mainly lymphocytes) can be seen.
B. In the right (piglet no. 2). Unlike to 1A, this Figure shows areas of thickened interalveolar septa due to the accumulation of mononucellular cells (mainly of lymphocytes). Such focal mild non-specific interstitial infiltrate (MNSII) was found in the lungs of 5 out of 9 uninfected controls (Table 1).

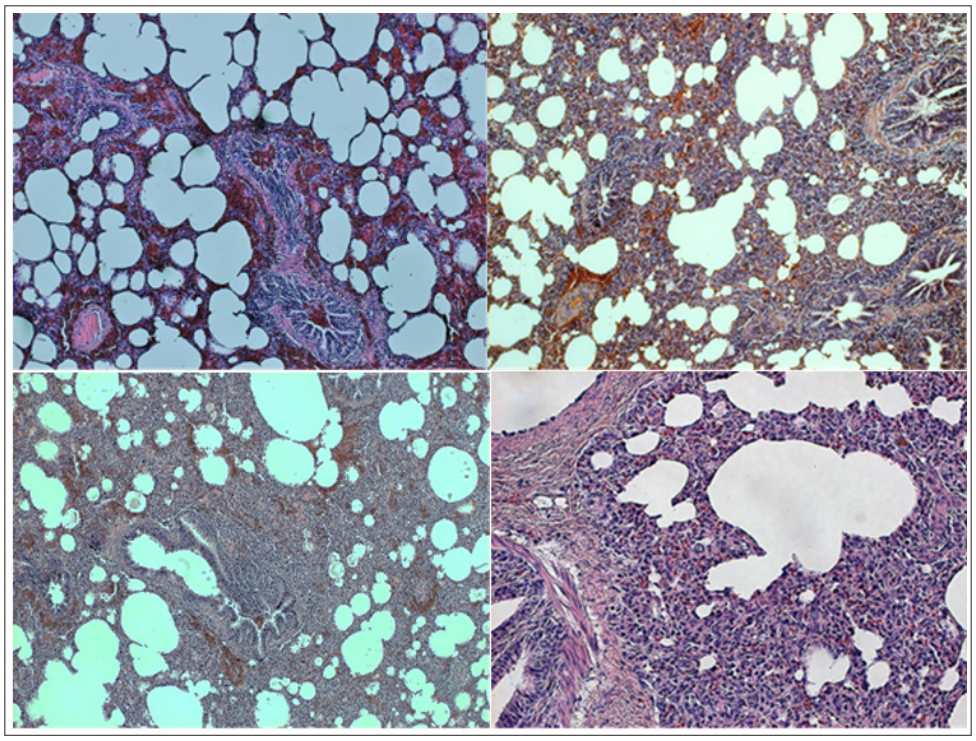

Figure 2: Histological findings in the lungs of PRRSV infected animals

A. In the left above (piglet no. 16). At low power view some areas of the lung tissue even in the infected animal showed rather less extensive thickening of interalveolar septa (infiltration by mononuclear cells referred to as mild non-specific interstitial infiltrate, MNSII); note the dilatation of small vessels (magn x100).
2B. In the right above (piglet no. 16). In contrast to the area shown above, another lung area of the same reveals typical UIP with more widespread thickening of interalveolar septa and their abundant mononuclear cell infiltration (along with hyperemia, i.e. dilatation of capillaries and small blood vessels).
2C. In the left below (piglet no. 25). The lung tissue of an animal who developed typical UIP shows widespread mononuclear infiltration of interalveolar septa and peribronchial connective tissue (a lymphatic follicle like structure can be seen, magn. x100).
2D. In the right below, the same piglet as above (no. 25). The mononuclear infiltrate in the peribronchial area consists mainly of lymphocytes along causes thickening of interalveolar septa (magn. x240).

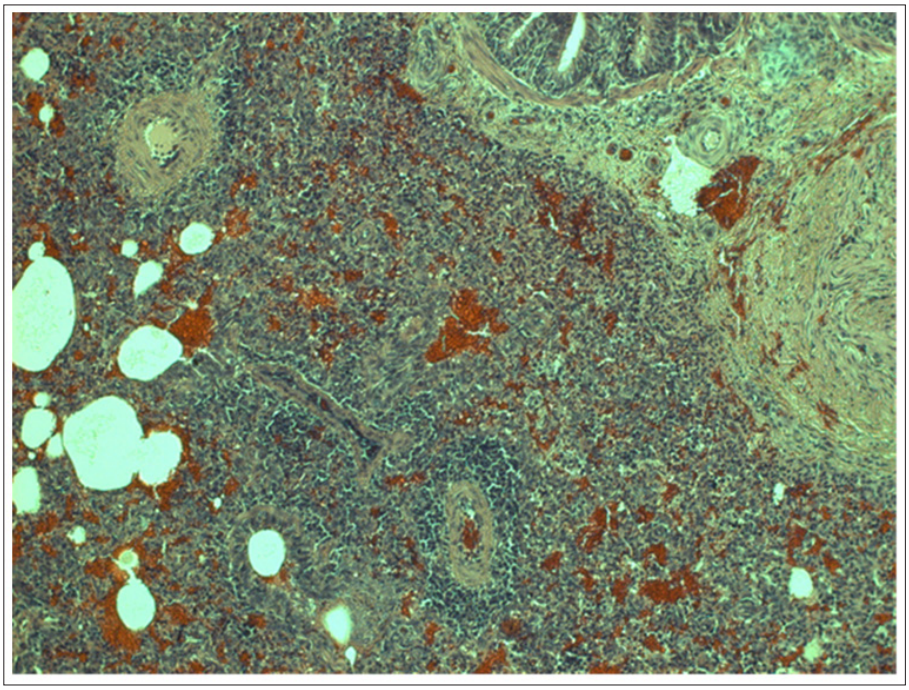

Figure 3: Extensive interstitial pneumonia due to PRRSV infection

The lungs of piglet no.40 show extremely severe interstial pneumonitis characterized by widespread infiltration of the septa by mononuclear cells mainly lymphocytes which replace the original lung structure; in addition, abundant hyperemia is present along with extensive proliferation of the connective (fibrous) tissue (magn.220x).

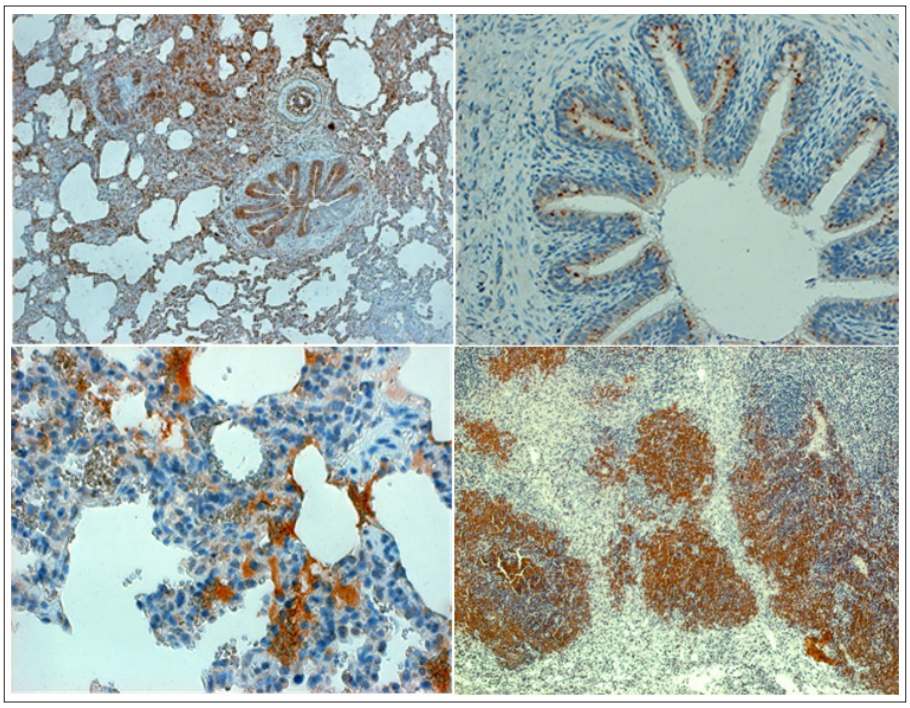

Figure 4: Staining for N-antigen in the respiratory tract and spleen

A. In the left above (piglet no. 34). The lungs of infected animals reveal overwhelming positive staining for N-protein, namely in the bronchial epithelium, in parabronchial mucinous glands and occasionally in the flat epithelium cells lining the aveoli (magn. 80x).
B. In the right above (piglet no. 12). The N-protein can be seen in the cytoplasm of ciliary epithelium cells lining the bronchi along with the negative goblet cells (magn. 120x).
C. In the left below (piglet no. 45). The N-protein can be seen in the cytoplasm of cells lining the alveolar wall and in mononuclear phagocytes which infiltrate the interalveolar septi (magn. 400x).
D. In the right below (animal no. 44): the spleen showing lymphatic follicles consisting mainly of lymphocytes positive for the N-protein (magn.x120).

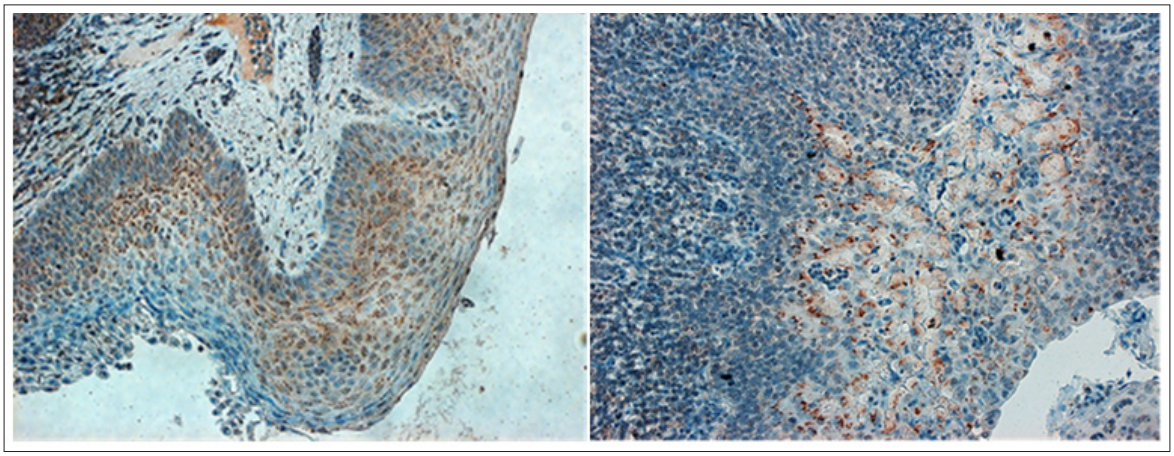

Figure 5: N-protein in the pharyngeal area of PRRSV infected animals

A. In the left (piglet no. 26). In the tonsillar squamous epithelium, the N-protein is expressed mainly wthin cytoplasm of actively growing cells of the suprabasal and intermedial layers including a few basal epithelium cells (magn. 220x)
B. In the right (pig no. 13). N-protein can be seen in the acini of a submandibular salivary gland as well as in the marginal sinus of adjacent lymph node (magn. 220x).

Table 1: Survey of histological lesions and the N-antigen presence in infected piglets

Animal	PRRSV	Results
Uninfected	None	MNSII* in the lungs (4/9), no N-antigen seen neither in bronchi (0/9) nor in tonsils (0/9)
Infected	Yes	In the lungs the prevalence of UIP**(23/28), less frequently MNSII (5/28); the N-antigen was seen in bronchial epithelium (21/28) and/or alveolar wall (5/28). Outside of lungs, the N-antigen was detected in tonsilar epithelium (13/28), spleen (3/28) and salivary gland (1/28).

*mild non-specific interstitial infiltrate in the peribronchial area and/or interalveolar septa
** severe interstitial infiltrate corresponding to the diagnosis of „Usual Interstitial Pneumonia" (for details see „The respiratory system", pp. 556-617, in: E. Rubin, Farber J.L. [eds], Pathology 2nd ed., Lippincott Company, Philadelphia, 1994)

Table 2: The comparison of UIP with serological response

Animal group	Histology		ELISA (antibody)	
	UIP	MNSII	on day 11	on day 18
Negative control	0/9	4/9	0/9	0/9
Positive infected	23/28 (82%)	5/28 (18%)	2/28 (7 %)	16/16 (100 %)

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