Diagnostic comparison of serum and EDTA-stabilized blood samples for the detection of foot-and-mouth disease virus RNA by RT-qPCR

Foot-and-mouth disease (FMD) remains a globally important disease but there have only been occasional recent outbreaks in Europe, e.g. in the U.K. in 2001, U.K. 2007 and Bulgaria 2010/2011. However, this infection still poses a threat to Europe as the disease occurs close to its borders and incursions can occur through importation of contaminated animal products and through the air. To deal with a suspected outbreak, fast sampling, transportation and accurate laboratory diagnosis are critical; testing for FMDV is normally performed on epithelium samples or serum. Assessment of the use of stabilized blood in assays for FMDV RNA is useful as this sample material can be prepared on site for safe transportation and rapid analysis at the laboratory. Such samples are also collected for diagnosis of other diseases giving similar clinical signs. Testing serum and EDTA-stabilized blood samples from FMDV-infected cattle and pigs, using real time quantitative RT-PCR assays, yielded similar results. However, detection of FMDV RNA was less sensitive (about 10-fold) when using EDTA-stabilized blood compared to serum. Thus, diagnosis of FMD can be achieved using EDTA-stabilized blood samples in an outbreak situation on a herd basis, but serum is preferred at the single animal level for optimal sensitivity.

Simulation of transmission and persistence of African swine fever in wild boar in Denmark

African swine fever (ASF) is caused by ASF virus (ASFV) and is currently circulating in the eastern part of Europe posing a serious risk regarding transmission to western European countries. Wild boar is a main driver of the transmission and persistence of ASFV in the endemic infected countries in Europe. Some European countries free from ASF, such as Denmark and the Netherlands, have limited population sizes of wild boar, but have large swine productions. In these countries, the patterns of transmission and persistence of ASFV in the existing wild boar population, in case of introduction of ASFV, are unknown. It is important to get a better understanding of ASFV in these wild boar populations, in order to better manage the existing wild boar population and thereby minimize the risk of virus introduction and transmission to domestic pigs, in case of an ASFV incursion. We created an agent-based spatio-temporal model and simulated the transmission of ASFV within Danish wild boar populations, using actual landscape data. The model was run with 50 and 100 wild boar groups used as initial population sizes, respectively, either distributed across the southern part of the mainland (Jutland) or across both the southern and middle parts of Jutland, where wild boar groups are believed to exist. At first, the model was run without ASFV for 25 years to assess wild boar population dynamics in both regions. Thereafter, ASFV was added to the model 1 year after initiation and run for up to another 4 years. The model predicted that wild boar populations may increase drastically over the next 25 years, if wild boar groups were distributed across both southern and middle Jutland and no mitigation actions were taken, while the population sizes will be restricted, if groups were distributed only across the southern part of Jutland. The density of the population is an important factor affecting the transmission and persistency of the disease. Model results indicated that ASF epidemics in the simulated populations would generally persist for few months. However, due to the high stochasticity of the process, in certain situations the epidemics may last for more than one year, posing a serious risk of ASFV introduction to domestic pigs.
A reply to "A comment on "Inter-laboratory study to characterize the detection of serum antibodies against porcine epidemic diarrhoea virus""

I have read with very much interest the paper of Strandbygaard et al. recently published in Veterinary Microbiology (2016, 197, 151–160). As a veterinary diagnostic laboratory Biovet recognizes the interest of such kind of study which evaluates the performances of diagnostic assays in various laboratories.

Complete genome sequence of an African swine fever virus (ASFV POL/2015/Podlaskie) determined directly from pig erythrocyte-associated nucleic acid

African swine fever (ASF) is an important disease of domestic pigs and wild boar. The disease is caused by African swine fever virus (ASFV). In 2014, ASFV was introduced into Eastern Europe, and it has since then continued to spread within various Eastern European countries. Investigating differences in sequences between ASFV isolates may be a valuable tool to understand differences in virulence among them, however currently, no complete genome sequences of the viruses responsible for the Eastern European outbreaks have been reported. In this study, the complete genome sequence of a highly virulent ASFV was determined directly from erythrocyte-associated nucleic acids obtained from a pig experimentally infected with an isolate from Poland (ASFV POL/2015/Podlaskie). The sequence (ca. 189 kb) of this recent European ASFV showed 95 nt differences (99.95 % identity) from the ASFV Georgia 2007/1 genome. The complete sequence of ASFV/Pol/2015/Podlaskie should assist further studies on the genetic diversity and evolution of the European ASFVs.
Detection and Characterization of Distinct Alphacoronaviruses in Five Different Bat Species in Denmark

Bat populations harbour a multitude of viruses; some of these are pathogenic or potentially pathogenic in other animals or humans. Therefore, it is important to monitor the populations and characterize these viruses. In this study, the presence of coronaviruses (CoVs) in different species of Danish bats was investigated using active surveillance at different geographical locations in Denmark. Faecal samples were screened for the presence of CoVs using pan-CoV real-time RT-PCR assays. The amplicons, obtained from five different species of bats, were sequenced. Phylogenetic analysis revealed a species-specific clustering with the samples from Myotis daubentonii, showing a close resemblance to coronavirus sequences obtained from the same species of bat in Germany and the United Kingdom. Our results show, for the first time, that multiple, distinct alphacoronaviruses are present in the Danish bat populations.
Efficacy and safety of simultaneous vaccination with two modified live virus vaccines against porcine reproductive and respiratory syndrome virus types 1 and 2 in pigs

The objective of the study was to compare responses of pigs vaccinated with a PRRS MLV vaccine against PRRSV-1 or PRRSV-2 with the responses of pigs vaccinated simultaneously with both vaccines. Furthermore, the efficacy of the two PRRSV MLV vaccination strategies was assessed following challenge. The experimental design included four groups of 4-weeks old SPF-pigs. On day 0 (DPV0), groups 1–3 (N = 18 per group) were vaccinated with modified live virus vaccines (MLV) containing PRRSV-1 virus (VAC-T1), PRRSV-2 virus (VAC-T2) or both (VAC-T1T2). One group was left unvaccinated (N = 12). On DPV 62, the pigs from groups 1–4 were mingled in new groups and challenged (DPC 0) with PRRSV-1, subtype 1, PRRSV-1, subtype 2 or PRRSV-2. On DPC 13/14 all pigs were necropsied. Samples were collected after vaccination and challenge. PRRSV was detected in all vaccinated pigs and the majority of the pigs were positive until DPV 28, but few of the pigs were still viremic 62 days after vaccination. Virus was detected in nasal swabs until DPV 7–14. No overt clinical signs were observed after challenge. PRRSV-2 vaccination resulted in a clear reduction in viral load in serum after PRRSV-2 challenge, whereas there was limited effect on the viral load in serum following challenge with the PRRSV-1 strains. Vaccination against PRRSV-1 had less impact on viremia following challenge. The protective effects of simultaneous vaccination with PRRSV Type 1 and 2 MLV vaccines and single PRRS MLV vaccination were comparable. None of the vaccines decreased the viral load in the lungs at necropsy. In conclusion, simultaneous vaccination with MLV vaccines containing PRRSV-1 and PRRSV-2 elicited responses comparable to single vaccination and the commercial PRRSV vaccines protected only partially against challenge with heterologous strains. Thus, simultaneous administration of the two vaccines is an option in herds with both PRRSV types.

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Full-length genome sequences of porcine epidemic diarrhoea virus strain CV777; Use of NGS to analyse genomic and sub-genomic RNAs

Porcine epidemic diarrhoea virus, strain CV777, was initially characterized in 1978 as the causative agent of a disease first identified in the UK in 1971. This coronavirus has been widely distributed among laboratories and has been passaged both within pigs and in cell culture. To determine the variability between different stocks of the PEDV strain CV777, sequencing of the full-length genome (ca. 28kb) has been performed in 6 different laboratories, using different protocols. Not surprisingly, each of the different full genome sequences were distinct from each other and from the reference sequence (Accession number AF353511) but they are >99% identical. Unique and shared differences between sequences were identified. The coding region for the surface-exposed spike protein showed the highest proportion of variability including both point mutations and small deletions. The predicted expression of the ORF3 gene product was more dramatically affected in three different variants of this virus through either loss of the initiation codon or gain of a premature termination codon. The genome of one isolate had a substantially rearranged 5’-terminal sequence. This rearrangement was validated through the analysis of sub-genomic mRNAs from infected cells. It is clearly important to know the features of the specific sample of CV777 being used for experimental studies.

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Infection of pigs with African swine fever virus via ingestion of stable flies (Stomoxys calcitrans)

Within Eastern Europe, African swine fever virus (ASFV) has unexpectedly spread to farms with high biosecurity. In an attempt to explain this process, pigs were allowed to ingest flies that had fed on ASFV-spiked blood, which had a realistic titre for an infected pig. Some of the pigs became infected with the virus. Thus, ingestion of blood-sucking flies, having fed on ASFV-infected wild boar before entering stables, represents a potential route for disease transmission.

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Modeling the Effects of Duration and Size of the Control Zones on the Consequences of a Hypothetical African Swine Fever Epidemic in Denmark

African swine fever (ASF) is a notifiable infectious disease. The disease is endemic in certain regions in Eastern Europe constituting a risk of ASF spread toward Western Europe. Therefore, as part of contingency planning, it is important to continuously explore strategies that can effectively control an epidemic of ASF. A previously published and well documented simulation model for ASF virus spread between herds was used to examine the epidemiologic and economic impacts of the duration and size of the control zones around affected herds. In the current study, scenarios were run, where the duration of the protection and surveillance zones were reduced from 50 and 45 days to 35 and 25 days or to 35 and 25 days, respectively. These scenarios were run with or without enlargement of the surveillance zone around detected herds from 10 to 15 km. The scenarios were also run with only clinical or clinical and serological surveillance of herds within the zones. Sensitivity analysis was conducted on influential input parameters in the model. The model predicts that reducing the duration of the protection and surveillance zones has no impact on the epidemiologic consequences of the epidemics, while it may result in a substantial reduction in the total economic losses. In addition, the model predicts that increasing the size of the surveillance zone from 10 to 15 km may reduce both the epidemic duration and the total economic losses, in case of large epidemics. The ranking of the control strategies by the total costs of the epidemics was not influenced by changes of input parameters in the sensitivity analyses.

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Short time window for transmissibility of African swine fever virus from a contaminated environment

Since the introduction of African swine fever virus (ASFV) into the Baltic states and Poland in 2014, the disease has continued to spread within these regions. In 2017, the virus spread further west and the first cases of disease were reported in the Czech Republic and Romania, in wild boar and domestic pigs, respectively. To control further spread, knowledge of different modes of transmission, including indirect transmission via a contaminated environment, is crucial. Up until now, such an indirect mode of transmission has not been demonstrated. In this study, transmission via an environment contaminated with excretions from ASFV-infected pigs was investigated. Following euthanasia of pigs that were infected with an isolate of ASFV from Poland (POL/2015/Podlaskie/Lindholm), healthy pigs were introduced into the pens, in which the ASFV-infected pigs had been housed. Introduction was performed at 1, 3, 5 or 7 days, following euthanasia of the infected pig groups. Pigs, that were introduced into the contaminated environment after 1 day, developed clinical disease within 1 week, and both ASFV DNA and infectious virus were isolated from their blood. However, pigs introduced into the contaminated pens after 3, 5 or 7 days did not develop any signs of ASFV infection and no viral DNA was detected in blood samples obtained from these pigs within the following 3 weeks. Thus, it was shown that exposure of pigs to an environment contaminated with ASFV can result in infection. However, the time window for transmissibility of ASFV seems very limited, and, within our experimental system, there appears to be a rapid decrease in the infectivity of ASFV in the environment.

Survival and localization of African swine fever virus in stable flies (Stomoxys calcitrans) after feeding on viremic blood using a membrane feeder

Since 2014, African swine fever virus (ASFV) has been spreading within Eastern Europe. Within affected regions, the virus has infected some farms with high biosecurity and a marked seasonality of outbreaks in domestic pigs has been observed. ASFV transmission from stable flies, Stomoxys calcitrans, has previously been shown both mechanically and via ingestion of whole flies. Hence, blood-feeding flies may offer one explanation for the introductions into high biosecurity farms and for the observed seasonality. The aim of this study was to further elucidate the potential role of stable flies in ASFV transmission. Different parts of flies were analyzed for the presence of viral DNA and infectious virus at different time points following in vitro feeding of the flies on blood from an ASFV-infected pig. Using qPCR, ASFV DNA was detectable in mouth parts of flies for at least 12 h and remained in head and body samples from the flies for up to three days following
Feeding. Infectious virus was detected in fly body samples prepared at 3 h and 12 h after feeding. The presence of infectious ASFV in stable flies following feeding on viremic blood means that such flies are capable of transporting infectious virus. The detection of ASFV DNA in the flies for up to three days following feeding suggests that qPCR analysis of blood-feeding flies during ASFV outbreaks could be a useful method to elucidate the role of these flies in ASFV transmission under field conditions.

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**Transmission of foot-and-mouth disease from persistently infected carrier cattle to naïve cattle via transfer of oropharyngeal fluid.**
Control and eradication of foot-and-mouth disease (FMD) is impeded by the existence of a persistent, subclinical, phase of infection in ruminants; animals with this status are referred to as carriers. However, the epidemiological significance of these FMD virus (FMDV) carriers is uncertain. In the current investigation, the contagion associated with FMDV carrier cattle was investigated by exposure of susceptible cattle and pigs to oropharyngeal fluid (OPF) or tissues harvested from persistently infected cattle. Naïve cattle were inoculated through intra nasopharyngeal deposition of unprocessed OPF that had been collected from FMDV carriers at 30 days post infection. These inoculated cattle developed clinical FMD of similar severity as animals that had been infected with a high-titer inoculum. In contrast, pigs exposed via intra oropharyngeal inoculation of the same OPF, or by ingestion of nasopharyngeal tissues harvested from the same cohort of persistently infected cattle, did not develop FMD. These findings indicate that there is demonstrable contagion associated with FMDV carrier cattle despite the lack of evidence for transmission by direct contact. The findings presented herein provide novel information that should be considered for FMD risk mitigation strategies.

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Outbreaks of porcine epidemic diarrhoea (PED) were reported across Europe during the 1980s and 1990s, but only sporadic outbreaks occurred in recent years. PED virus (PEDV) spread for the first time into the USA in 2013 and has caused severe economic losses. Retrospectively, it was found that two different strains of PEDV have been introduced into the United States, both are closely related to strains circulating in China where a new wave of the disease occurred from 2010 onwards. Since autumn 2014, new outbreaks of PED have occurred in Europe. In this study, weaned piglets were inoculated with an early European isolate (Br1/87) or faecal/intestinal suspensions derived from pigs infected with a recent European strain of PEDV (from Germany) or a US strain of PEDV. No evidence for infection resulted from inoculation of pigs with the German sample that contained high levels of PEDV RNA; there were no clinical signs, excretion of viral RNA or anti-PEDV antibody production. In contrast, all the pigs in the other two groups showed evidence of infection. Mild clinical signs of disease, mainly diarrhoea, occurred in piglets inoculated with the Br1/87 and US PEDV strains. PEDV RNA was detected throughout the intestine in euthanized animals at 4 days post-inoculation. In addition, within these animals, low levels of viral RNA were detected in lungs and livers with higher levels in spleens. Seroconversion against PEDV occurred in all surviving infected animals within 10 days. PEDV RNA excretion occurred for at least 2 weeks. The US PEDV RNA was detected at low levels in serum samples on multiple days. It is apparent that current diagnostic systems can detect infection by the different virus strains.

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Genetic and biological characterization of a Porcine Reproductive and Respiratory Syndrome Virus 2 (PRRSV-2)causing significant clinical disease in the field
Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is the cause of severe reproductive and respiratory disease in swine worldwide. In Denmark, both PRRSV-1 and PRRSV-2 are circulating and approximately 35% of pig herds are seropositive for PRRSV. In November 2010, a pig herd in the Northern part of Denmark experienced an
infection with PRRSV-2 with clinical signs that were much more severe than normally reported from current Danish
PRRSV-2 affected herds. Due to the clinical observations of reproductive failure in sows and high mortality in piglets, it
was speculated that a new, more pathogenic or vaccine evading PRRS strain had emerged in Denmark. The overall aim
of the present study was to perform a genetic and biological characterization of the virus isolated from the diseased herd.
Complete genome sequencing of isolates from this herd revealed that although the case strain had some unique genetic
features including a deduced 3 amino acid deletion, it was in overall very similar to the other PRRS-2 viruses circulating in
Denmark. In an experimental trial in growing pigs, no overt clinical signs or pathology were observed following intranasal
inoculation with the new virus isolate. Virus shedding, acute phase protein responses and serological responses were
comparable to those seen after experimental challenge with a Danish PRRS-2 reference strain isolated in 1997.
Vaccination with a commercial modified live PRRSV-2 vaccine had a clear reducing effect on virus shedding, magnitude,
and duration of viremia and viral load in the lungs. Overall, the results indicate that the severe disease observed in the
field was contributed by additional factors in combination with the PRRS virus infection.

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Pathogenicity of three genetically diverse strains of PRRSV Type 1 in specific pathogen free pigs
Studies from Eastern European countries proved that porcine reproductive and respiratory syndrome virus Type 1
(DecOxV-1) harbours high genetic diversity and that genetically divergent subtypes 2-4 circulate in this area. In the present
study, we compared the pathogenicity of two different DecOxV-1 subtype 2 strains and a strain representing DecOxV-1
subtype 1. Four groups of 8-week-old specific pathogen free pigs were either infected with subtype 2 strain ILI6, subtype 2
strain or BOR59, subtype 1 strain 18794, or mock inoculated. The most pronounced clinical signs were observed in pigs
infected with BOR59. Pigs from both subtype 2 strain infected groups exhibited significantly elevated mean body
temperatures on DPI 2 compared to the other two groups, the difference remaining significant up to DPI 13 for the BOR59
group, only. The pigs in the latter group also displayed significantly highest levels of early viremia together with the most
rapid APP response. Overall, the results indicated that BOR59 strain can be considered a highly pathogenic strain,
similarly to subtype 3 strains Lena and SU1-bel, while the virulence of the other subtype 2 strain ILI6 was intermediate
between BOR59 and subtype 1 strain.

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Hjulsager, C. K., Huc, T., Kvisgaard, L. K., Sapierzyński, R., Nielsen, J.
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Transmission of African swine fever virus from infected pigs by direct contact and aerosol routes

In 2014, African swine fever virus (ASFV) was introduced into the Baltic states and Poland. Since then, the disease has continued to spread within these regions, and recently, cases were reported in the Czech Republic and Romania. Currently, there is an increasing risk of ASFV introduction into Western Europe. Hence, there is an urgent need to assess current contingency plans. For this purpose, knowledge of modes-of-transmission and clinical outcome in pigs infected with new European ASFV strains is needed. In the present study, two experiments were conducted in pigs using an isolate of ASFV from Poland (designated here POL/2015/Podlaskie/Lindholm). In both studies, pigs were inoculated intranasally with the virus and contact pigs were exposed to the experimentally infected pigs, either directly (contact within and between pens) or by air. Pigs exposed to the virus by intranasal inoculation, by direct contact to infected animals and by aerosol developed acute disease characterized by viremia, fever and depression. Infectious virus was first detected in blood obtained from the inoculated pigs and then sequentially among the within-pen, between-pen and air-contact pigs. ASFV DNA and occasionally infectious virus was found in nasal-, oral-, and rectal swabs obtained from the pigs, and ASFV DNA was detected in air samples. No anti-ASFV antibodies were detected in sera. In conclusion, the study shows that the currently circulating strain of ASFV can be efficiently transmitted via direct contact and by aerosols. Also, the results provide quantitative transmission parameters and knowledge of infection stages in pigs infected with this ASFV.

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A Prime-Boost Vaccination Strategy in Cattle to Prevent Foot-and-Mouth Disease Using a "Single-Cycle" Alphavirus Vector and Empty Capsid Particles

Foot-and-mouth disease (FMD) remains one of the most economically important infectious diseases of production animals globally. Vaccination can successfully control this disease, however, current vaccines are imperfect. They are made using chemically inactivated FMD virus (FMDV) that is produced in large-scale mammalian cell culture under high containment conditions. Here, we have expressed the FMDV capsid protein precursor (P1-2A) of strain O1 Manisa alone or with the FMDV 3C protease (3Cpro) using a "single cycle" packaged alphavirus self-replicating RNA based on Semliki Forest virus (SFV). When the FMDV P1-2A was expressed with 3Cpro then processing of the FMDV capsid precursor protein is observed within cells and the proteins assemble into empty capsid particles. The products interact with anti-FMDV antibodies in an ELISA and bind to the integrin αvβ6 (a cellular receptor for FMDV). In cattle vaccinated with these rSFV-FMDV vectors alone, anti-FMDV antibodies were elicited but the immune response was insufficient to give protection against FMDV challenge. However, the prior vaccination with these vectors resulted in a much stronger immune response against FMDV post-challenge and the viremia observed was decreased in level and duration. In subsequent experiments, cattle were sequentially vaccinated with a rSFV-FMDV followed by recombinant FMDV empty capsid particles, or vice versa, prior to challenge. Animals given a primary vaccination with the rSFV-FMDV vector and then boosted with FMDV empty capsids showed a strong anti-FMDV antibody response prior to challenge, they were protected against disease and no FMDV RNA was detected in their sera post-challenge. Initial inoculation with empty capsids followed by the rSFV-FMDV was much less effective at combating the FMDV challenge and a large post-challenge boost to the level of anti-FMDV antibodies was observed. This prime-boost system, using reagents that can be generated outside of high-containment facilities, offers significant advantages to achieve control of FMD by vaccination.

General information

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Assessing the potential spread and maintenance of foot-and-mouth disease virus infection in wild ungulates: general principles and application to a specific scenario in Thrace

Foot-and-mouth disease (FMD), due to infection with serotype O virus, occurred in wild boar and within eleven outbreaks in domestic livestock in the south-east of Bulgaria, Thrace region, in 2011. Hence, the issue of the potential for the spread and maintenance of FMD virus (FMDV) infection in a population of wild ungulates became important. This assessment focused on the spread and maintenance of FMDV infection within a hypothetical wild boar and deer population in an environment, which is characterized by a climate transitional between Mediterranean and continental and variable wildlife population densities. The assessment was based on three aspects: (i) a systematic review of the literature focusing on experimental infection studies to identify the parameters describing the duration of FMDV infection in deer and wild boar, as well as observational studies assessing the occurrence of FMDV infection in wild deer and wild boar populations, (ii) prevalence survey data of wild boar and deer in Bulgaria and Turkey and (iii) an epidemiological model, simulating the host-to-host spread of FMDV infections. It is concluded, based on all three aspects, that the wildlife population in Thrace, and so wildlife populations in similar ecological settings, are probably not able to maintain FMD in the long term in the absence of FMDV infection in the domestic host population. However, limited spread of FMDV infection in time and space in the wildlife populations can occur. If there is a continued crossover of FMDV between domestic and wildlife populations...
or a higher population density, virus circulation may be prolonged.

Characterization of a Novel Chimeric Swine Enteric Coronavirus from Diseased Pigs in Central Eastern Europe in 2016

During a severe outbreak of diarrhoea and vomiting in a pig herd in Central Eastern Europe, faecal samples were tested positive for porcine epidemic diarrhoea virus (PEDV) and negative for transmissible gastroenteritis virus (TGEV) using a commercial RT-qPCR assay that can detect both of these coronaviruses. However, further analyses, using other TGEV- and PEDV-specific RT-qPCR assays, provided results inconsistent with infection by either of these viruses. Sequencing of an amplicon (ca. 1.6 kb), generated by an RT-PCR specific for the PEDV S-gene, indicated a very close similarity (ca. 99% identity) to recently described chimeric viruses termed swine enteric coronaviruses (SeCoVs). These viruses (with an RNA genome of ca. 28 kb) were first identified in Italy in samples from 2009 but have not been detected there since 2012. A closely related virus was detected in archived samples in Germany from 2012, but has not been detected subsequently. Building on the initial sequence data, further amplicons were generated and over 9 kb of sequence corresponding to the 3′-terminus of the new SeCoV genome was determined. Sequence comparisons showed that the three known SeCoVs are ≥98% identical across this region and contain the S-gene and 3a sequences from PEDV within a backbone of TGEV, but the viruses are clearly distinct from each other. It is demonstrated, for the first time, that pigs from within the SeCoV-infected herd seroconverted against PEDV but tested negative in a TGEV-specific ELISA that detects antibodies against the S protein. These results indicate that SeCoV is continuing to circulate in Europe and suggest it can cause a disease that is very similar to PED. Specific detection of the chimeric SeCoVs either requires development of a new diagnostic RT-qPCR assay or the combined use of assays targeting the PEDV S-gene and another part of the TGEV genome.
Control of African swine fever epidemics in industrialized swine populations

African swine fever (ASF) is a notifiable infectious disease with a high impact on swine health. The disease is endemic in certain regions in the Baltic countries and has spread to Poland constituting a risk of ASF spread toward Western Europe. Therefore, as part of contingency planning, it is important to explore strategies that can effectively control an epidemic of ASF. In this study, the epidemiological and economic effects of strategies to control the spread of ASF between domestic swine herds were examined using a published model (DTU-DADS-ASF). The control strategies were the basic EU and national strategy (Basic), the basic strategy plus pre-emptive depopulation of neighboring swine herds, and intensive surveillance of herds in the control zones, including testing live or dead animals. Virus spread via wild boar was not modelled.

Under the basic control strategy, the median epidemic duration was predicted to be 21 days (5th and 95th percentiles; 1-55 days), the median number of infected herds was predicted to be 3 herds (1–8), and the total costs were predicted to be €326 million (€256–€442 million). Adding pre-emptive depopulation or intensive surveillance by testing live animals resulted in marginal improvements to the control of the epidemics. However, adding testing of dead animals in the protection and surveillance zones was predicted to be the optimal control scenario for an ASF epidemic in industrialized swine populations without contact to wild boar. This optimal scenario reduced the epidemic duration to 9 days (1–38) and the total costs to €294 million (€257–€392 million). Export losses were the driving force of the total costs of the epidemics.

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Diagnostic evaluation of assays for detection of antibodies against porcine epidemic diarrhea virus (PEDV) in pigs exposed to different PEDV strains

Porcine epidemic diarrhea virus (PEDV) has caused economic losses in the Americas, Asia and Europe in recent years. Reliable serological assays are essential for epidemiological studies and vaccine evaluation. The objective of this study was to compare the ability of five enzyme-linked immunosorbent assays (ELISAs) to detect antibodies against different PEDV strains in pig serum. A total of 732 serum samples from North American or European pigs were tested. Samples included experimental samples from pigs infected with classical (G1a PEDV) or variant genogroup 1 PEDV (G1b PEDV),
pandemic genogroup 2 PEDV (G2b PEDV) or non-infected controls. Field samples from herds with confirmed or unknown PEDV exposure were also used. Three indirect ELISAs based on G2b antigens (ELISAs 1, 2 and 3), a competitive ELISA based on the G2b antigen (ELISA 4) and a competitive ELISA based on the G1a antigen (ELISA 5) were compared. Overall, the tests had a moderate agreement (κ = 0.61). G1a PEDV infected pigs were earliest detected by ELISA 3, G1b PEDV infected pigs were earliest detected by ELISAs 4 and 5 and the performance of all tests was similar for the G2b PEDV group. ELISA 1 showed the overall lowest detection on experimentally and field derived samples. Diagnostic sensitivity and specificity with a 95% probability interval were estimated to be 68.2% (62.1–74.4%) and 97.5% (95.2–99.0%) for ELISA 1, 73.7% (71.5–76.6%) and 98.4% (96.6–99.5%) for ELISA 2, 86.2% (81.1–90.6%) and 91.6% (87.7–94.8%) for ELISA 3, 78.3% (72.8–83.5%) and 99.7% (98.2–100%) for ELISA 4, and 93.5% (90.3–96.0%) and 91.2% (83.8–97.9%) for ELISA 5. Differences in detection among assays seem to be more related to intrinsic factors of an assay than to the PEDV antigen used.

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Different capabilities of five ELISAs for detection of antibodies against PEDV in pigs exposed to geographically different strains

**General information**

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Experimental infection of piglets with an early European strain of PED virus and a recent US PEDV strain

**General information**

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Experimental pig-to-pig transmission study with a recent European African Swine Fever virus isolate

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Inter-laboratory study to characterize the detection of serum antibodies against porcine epidemic diarrhoea virus

Porcine epidemic diarrhea virus (PEDV) has caused extensive economic losses to pig producers in many countries. It was recently introduced, for the first time, into North America and outbreaks have occurred again in multiple countries within Europe as well. To assess the properties of various diagnostic assays for the detection of PEDV infection, multiple panels of porcine sera have been shared and tested for the presence of antibodies against PEDV in an inter-laboratory ring trial. Different laboratories have used a variety of “in house” ELISAs and also one commercial assay. The sensitivity and specificity of each assay has been estimated using a Bayesian analysis applied to the ring trial results obtained with the different assays in the absence of a gold standard. Although different characteristics were found, it can be concluded that each of the assays used can detect infection of pigs at a herd level by either the early European strains of PEDV or the recently circulating strains (INDEL and non-INDEL). However, not all the assays seem suitable for demonstrating freedom from disease in a country. The results from individual animals, especially when the infection has occurred within an experimental situation, show more variation.

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Occurrence of Swine Enteric Coronavirus (SeCoV) Infection during 2016 within Central Eastern Europe

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Bibliographical note
Poster 13

Prevention of foot-and-mouth disease in cattle using a prime-boot-vaccination strategy

Foot-and-mouth disease (FMD) is one of the most economically important infectious diseases of production animals globally. Vaccination can help to control this disease, however, current vaccines are imperfect. They are made using chemically inactivated FMD virus (FMDV) that is produced in mammalian cell culture under high containment. Here, we have expressed the FMDV capsid protein precursor (P1-2A) of strain O1 Manisa alone or with the FMDV 3C protease (3Cpro) using a "single cycle" packaged alphavirus self-replicating RNA based on Semliki Forest virus (SFV). When the FMDV P1-2A was expressed with 3Cpro then processing of the FMDV capsid precursor protein is observed within cells and the proteins assemble into empty capsid particles. In cattle vaccinated once with these rSFV-FMDV vectors alone, anti-FMDV antibodies were elicited but the immune response was insufficient to give protection against FMDV challenge. However, the prior vaccine with these vectors resulted in a much stronger immune response against FMDV post-challenge and the viremia observed was decreased in level and duration. In subsequent experiments, cattle were sequentially vaccinated with a rSFV-FMDV followed by recombinant FMDV empty capsid particles, or vice versa, prior to challenge. Animals given a primary vaccination with the rSFV-FMDV vector and then boosted with FMDV empty capsids showed a strong anti-FMDV antibody response prior to challenge. Following challenge with FMDV, the cattle were protected against disease and no FMDV RNA was detected in their sera. Initial inoculation with empty capsids followed by the rSFV-FMDV was much less effective at combating the FMDV challenge and a large post-challenge boost to the level of anti-FMDV antibodies was observed and clinical disease occurred. This prime-boost system, using reagents that can be generated outside of high-containment facilities, offers significant advantages to achieve control of FMD by vaccination.

QA prime-boost vaccination strategy in prevent serotype O FMDV infection using a "single-cycle" alphavirus vector and empty capsid particles

Introduction
Foot-and-mouth disease (FMD) remains one of the most economically important infectious diseases of production animals globally. Vaccination can help to control this disease, however, current vaccines based on chemically inactivated FMDV, are imperfect and there is a need for new, safe and effective vaccines to control FMD. There is no cross protection between the 7 serotypes but serotype O is the most abundant globally.
Material and methods
The FMDV capsid protein precursor (P1-2A) of strain O1 Manisa has been expressed with the FMDV 3C protease (3Cpro) using a “single cycle” packaged alphavirus self-replicating RNA based on Semliki Forest virus (SFV). Purified O1 Manisa empty capsid particles (ECs) have been prepared using a recombinant vaccinia virus expression system. Cattle have been vaccinated with the SFV-FMDV vectors and boosted subsequently with the ECs and then challenged with serotype O FMDV. The immune response against FMDV achieved by vaccination and infection status following challenge has been determined.

Results
In cattle vaccinated once with these rSFV-FMDV vectors alone, anti-FMDV antibodies were elicited but the immune response was insufficient to give protection against FMDV challenge. However, the vaccination with these vectors resulted in a much stronger immune response against FMDV post-challenge than in naïve animals. In subsequent experiments, cattle were sequentially vaccinated with the rSFV-FMDV followed by recombinant FMDV empty capsid particles prior to challenge. Animals given a primary vaccination with the rSFV-FMDV vector and then boosted with FMDV empty capsids showed a strong anti-FMDV antibody response prior to challenge. Following challenge with serotype O FMDV, the cattle were protected against disease and no FMDV RNA was detected in their sera.

Discussion
This prime-boost system, using reagents that can be generated outside of high-containment facilities, offers significant advantages to achieve control of FMD by vaccination.

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Simulating the epidemiological and economic effects of an African swine fever epidemic in industrialized swine populations
African swine fever (ASF) is a notifiable infectious disease with a considerable impact on animal health and is currently one of the most important emerging diseases of domestic pigs. ASF was introduced into Georgia in 2007 and subsequently spread to the Russian Federation and several Eastern European countries. Consequently, there is a non-negligible risk of ASF spread towards Western Europe. Therefore it is important to develop tools to improve our understanding of the spread and control of ASF for contingency planning. A stochastic and dynamic spatial spread model (DTU-DADS) was adjusted to simulate the spread of ASF virus between domestic swine herds exemplified by the Danish swine population. ASF was simulated to spread via animal movement, low- or medium-risk contacts and local spread. Each epidemic was initiated in a randomly selected herd – either in a nucleus herd, a sow herd, a randomly selected herd or in multiple herds simultaneously. A sensitivity analysis was conducted on input parameters. Given the inputs and assumptions of the model, epidemics of ASF in Denmark are predicted to be small, affecting about 14 herds in the worst-case scenario. The duration of an epidemic is predicted to vary from 1 to 76 days. Substantial economic damages are predicted, with median direct costs and export losses of €12 and €349 million, respectively, when epidemics were initiated in multiple herds. Each infectious herd resulted in 0 to 2 new infected herds varying from 0 to 5 new infected herds, depending on the index herd type.

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Simulation of Spread of African Swine Fever, Including the Effects of Residues from Dead Animals

To study the spread of African swine fever (ASF) within a pig unit and the impact of unit size on ASF spread, a simulation model was created. In the model, an animal can be in one of the following stages: susceptible, latent, subclinical, clinical, or recovered. Animals can be infectious during the subclinical stage and are fully infectious during the clinical stage. ASF virus (ASFV) infection through residues of dead animals in the slurries was also modeled in an exponentially fading-out pattern. Low and high transmission rates for ASFV were tested in the model. Robustness analysis was carried out in order to study the impact of uncertain parameters on model predictions. The results showed that the disease may fade out within the pig unit without a major outbreak. Furthermore, they showed that spread of ASFV is dependent on the infectiousness of subclinical animals and the residues of dead animals, the transmission rate of the virus, and importantly the unit size. Moreover, increasing the duration of the latent or the subclinical stages resulted in longer time to disease fade out. The proposed model is a simple and robust tool simulating the spread of ASFV within a pig house taking into account dynamics of ASFV spread and the unit size. The tool can be implemented in simulation models of ASFV spread between herds.

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Spatio-temporal Analysis of the Genetic Diversity of Arctic Rabies Viruses and Their Reservoir Hosts in Greenland

There has been limited knowledge on spatio-temporal epidemiology of zoonotic arctic fox rabies among countries bordering the Arctic, in particular Greenland. Previous molecular epidemiological studies have suggested the occurrence of one particular arctic rabies virus (RABV) lineage (arctic-3), but have been limited by a low number of available samples preventing in-depth high resolution phylogenetic analysis of RABVs at that time. However, an improved knowledge of the evolution, at a molecular level, of the circulating RABVs and a better understanding of the historical perspective of the disease in Greenland is necessary for better direct control measures on the island. These issues have been addressed by investigating the spatio-temporal genetic diversity of arctic RABVs and their reservoir host, the arctic fox, in Greenland using both full and partial genome sequences. Using a unique set of 79 arctic RABV full genome sequences from Greenland, Canada, USA (Alaska) and Russia obtained between 1977 and 2014, a description of the historic context in relation to the genetic diversity of currently circulating RABV in Greenland and neighboring Canadian Northern territories has been provided. The phylogenetic analysis confirmed delineation into four major arctic RABV lineages (arctic 1–4) with viruses from Greenland exclusively grouping into the circumpolar arctic-3 lineage. High resolution analysis enabled distinction of seven geographically distinct subclades (3.I – 3.VII) with two subclades containing viruses from both Greenland and Canada. By combining analysis of full length RABV genome sequences and host derived sequences encoding mitochondrial proteins obtained simultaneously from brain tissues of 49 arctic foxes, the interaction of viruses and their hosts was explored in detail. Such an approach can serve as a blueprint for analysis of infectious disease dynamics and virus-host interdependencies. The results showed a fine-scale spatial population structure in Greenland.
arctic foxes based on mitochondrial sequences, but provided no evidence for independent isolated evolutionary
development of RABV in different arctic fox lineages. These data are invaluable to support future initiatives for arctic fox
rabies control and elimination in Greenland.

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Contributors: Hanke, D., Freuling, C. M., Fischer, S., Hueffer, K., Hundertmark, K., Nadin-Davis, S., Marston, D., Fooks, A.
R., Bøtner, A., Mettenleiter, T. C., Beer, M., Rasmussen, T. B., Müller, T. F., Höper, D.
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**West Nile fever: En virussygdom, der spreder sig i Europa**
Status over West Nile virus i Europa og det danske overvågningsprogram.

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**Bat Coronaviruses circulating in Danish bats**
Bat Coronaviruses circulating in Danish bats

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Contributors: Rasmussen, T. B., Chriél, M., Baagøe, H. J., Fjederholt, E., Kooi, E. A., Belsham, G., Bøtner, A.
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Hvordan ser afrikansk svinepest ud i danske grise II? Rapport over smitteforsøg i drægtige søer 2014

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Use of recombinant capsid proteins in the development of a vaccine against foot-and-mouth disease virus (FMDV).

Foot-and-mouth disease remains one of the world’s most economically important diseases of livestock. It is caused by foot-and-mouth disease virus, a member of the picornavirus family. The virus replicates very rapidly and can be efficiently transmitted between hosts by a variety of routes. The disease has been effectively controlled in some parts of the world but remains endemic in many others, thus there is a constant risk of introduction of the disease into areas that are normally free of foot-and-mouth disease with potentially huge economic consequences. To reduce the need for large-scale culling of infected, and potentially infected, animals there has been significant effort to develop new vaccines against this disease which avoid some, or all, of the deficiencies of current vaccines. A major focus has been on the use of systems that express the structural proteins of the virus that self-assemble to generate “empty capsid” particles which share many features with the intact virus but lack the ribonucleic acid genome and are therefore non-infectious. Such particles can be “designed” to improve their stability or modify their antigenicity and can be produced without “high containment” facilities. The development and use of such improved vaccines should assist in the global efforts to control this important disease.
Characteristics of a foot-and-mouth disease virus with a partial VP1 G-H loop deletion in experimentally infected cattle

Previous work in cattle illustrated the protective efficacy and negative marker potential of a A serotype foot-and-mouth disease virus (FMDV) vaccine prepared from a virus lacking a significant portion of the VP1 G-H loop (termed A(−)). Since this deletion also includes the arginine-glycine-aspartate (RGD) motif required for virus attachment to the host cell in vivo, it was hypothesised that this virus would be attenuated in naturally susceptible animals. The A(−) virus was passaged three times in cattle via needle inoculation of virus suspension delivered into the intradermal space of the tongue (intradermolingual: IDL). Included in the study were three direct contact cattle, two of which were used for the third cattle passage (by inoculation) after direct contact exposure for three days. Cattle were monitored for clinical signs and samples were collected for sequencing as well as antibody and viral genome detection by ELISA and qRT-PCR. Following needle inoculation with the A(−) virus, naïve cattle developed typical clinical signs of FMDV infection, diagnostic assays also provided positive serological and virological results. However, the contact cattle did not develop clinical signs or generate serological or virological markers indicative of FMDV infection even when the cattle were subsequently needle inoculated with 105 TCID50 A(−) FMDV delivered IDL following three days of direct contact exposure. The results suggest that the A(−) virus is not attenuated in cattle when inoculated IDL. This virus could be useful as a tool to understand further the natural pathogenesis, receptor usage and internalisation pathways of FMDV.

Clinical characterization of a type 2 PRRSV causing significant clinical disease in the field in Denmark

General information

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Detection of European bat lyssavirus type 2 in Danish Daubenton’s bats

European bat lyssavirus (EBLV) is considered to be endemic in the Danish bat populations, but limited information exists about the types of EBLV strains currently in circulation. EBLV type 1 (EBLV-1) is seen as the predominant type in the Serotine bats (Eptesicus serotinus) with the latest case identified in 2009.


In the last decade, many porcine epidemic diarrhoea (PED) outbreaks have been reported by several countries in Asia whereas only a few Member States of the European Union (EU) have reported PED clinical cases and/or PED virus (PEDV)-seropositive animals. This alphacoronavirus was first reported in the USA in May 2013, followed by rapid spread throughout the country and outbreaks reported by several countries in the Americas. The recent PEDV-EU isolates have high level of sequence identity to PEDV-Am isolates. Based on nucleotide sequencing, multiple variants of PEDV are circulating in Europe, the Americas and Asia but any difference in virulence and antigenicity is currently unknown. Serological cross-reactivity has been reported between PEDV isolated in Europe and in the Americas; however no data regarding cross-protection are available. The impact of different PEDV strains is difficult to compare between one country and another, since impact is dependent not only on pathogenicity but also on factors such as biosecurity, farm management, sanitary status or herd immune status. However, the clinical signs of PEDV infections in naive pigs are similar in different countries with mortalities up to 100% in naive newborn piglets. The impact of recently reported PED outbreaks in Asia and the USA seems to be more severe than what has been described in Europe. Infected animals, faeces, feed and objects contaminated with faeces are matrices that have been reported to transmit PEDV between farms. Infectious PEDV has been detected in spray-dried porcine plasma (SDPP) in one study but the origin of the infectious PEDV in SDPP is not clear. Detection of porcine deltacoronavirus (PDCoV) has been reported in a few countries but only limited testing has been done. Based on the currently available information, it seems that PDCoV would have a lower impact than PEDV.
Experimental infection of pregnant sows with African swine fever (ASFV Georgia 2007): Clinical outcome, pathogenesis and vertical transmission

African swine fever virus (ASFV) causes a severe hemorrhagic fever in domestic pigs. The disease was introduced from the African continent to Georgia in 2007 and has since spread throughout the Caucasus and the Russian Federation. ASF is now established in Eastern Europe and outbreaks have occurred in domestic pigs and wild boar in Poland and the Baltic countries in 2014. Therefore, there is an increased risk of further transmission across Europe. The present study investigates the properties and the effect of the circulating ASF virus strain in Danish pregnant sows

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Limited interlaboratory comparison of Schmallenberg virus antibody detection in serum samples

Eight veterinary institutes in seven different countries in Europe participated in a limited interlaboratory comparison trial to evaluate laboratory performances of Schmallenberg virus (SBV) antibody detection in serum. Seven different sheep sera and three different cattle sera were circulated, and all participating institutes were asked to test these sera using SBV antibody detection assay(s) in place in their laboratories. All laboratories within the trial performed a virus neutralisation test (VNT) as well as one or two ELISAs on all samples, and swiftly detected SBV antibodies using these assays. VNT was more sensitive in detecting SBV antibodies than several of the used ELISA assays. Based on the test results, one cattle and one sheep SBV antibody-positive serum were selected to serve as reference sera, which now can be supplied to other laboratories on request.

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Rapid Spread of Schmallenberg Virus-infected Biting Midges (Culicoides spp.) across Denmark in 2012

Detection of Schmallenberg virus RNA, using real-time RT-PCR, in biting midges (Culicoides spp.) caught at 48 locations in 2011 and four well-separated farms during 2012 in Denmark, revealed a remarkably rapid spread of virus-infected midges across the country. During 2012, some 213 pools of obsoletus group midges (10 specimens per pool) were examined, and of these, 35 of the 174 parous pools were Schmallenberg virus RNA positive and 11 of them were positive in the heads. Culicoides species-specific PCRs identified both C. obsoletus and C. dewulfi as vectors of Schmallenberg virus.

Targeted modifications of foot-and-mouth disease virus; towards improved vaccine candidates

Foot-and-mouth disease virus (FMDV) is responsible for one of the most economically important diseases of farm animals (estimated annual costs are about US$10 billion globally). The virus is the prototypic Aphthovirus within the family Picornaviridae and has a positive sense RNA genome (ca. 8.3kb) encoding a single large polyprotein that is processed to generate about 15 mature proteins plus precursors. The virus particle comprises 60 copies of 4 separate capsid proteins (VP1-VP4) plus a single copy of the genome. By modifying full length cDNAs, producing RNA transcripts in vitro, and introducing these into susceptible cells it is possible to rescue specifically altered FMDVs. We have used this approach to generate modified viruses that have particular properties; these studies can assist in the development of improved and safer vaccines to protect against FMDV. For example, we have made changes to the leader (L) protein coding sequence. The L protein is the first component of the viral polyprotein and is produced in two forms, termed Lab and Lb as the result of use of alternative initiation codons, 84 nt apart. Both forms have protease activity (which separates the L protein from the capsid precursor) and induce the shut-off of host cell protein synthesis. When the shorter form, Lb, is precisely deleted...
then FMD viruses that grow well in cell culture are produced (Belsham, 2013). However such viruses are attenuated within cattle. In contrast, when the entire Lab coding sequence is deleted then no viable viruses are generated. In an alternative approach, we have modified a processing site within the viral polyprotein so that incomplete processing occurs. It has been shown that a single amino acid substitution that blocks cleavage of the VP1/2A junction within the capsid precursor results in the production of modified “self-tagged” virus particles that contain the VP1-2A precursor (Gullberg et al., 2013). This approach works for two of the most common FMDV serotypes (O and A) and offers the possibility of a single approach to purifying virus particles from different serotypes using reagents targeted to the conserved 2A peptide.

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The diagnostic utility of stabilized blood for detection of foot-and-mouth disease virus RNA by RT-qPCR
In Europe, clinical signs indicative of foot-and-mouth disease (FMD), would immediately lead to collection of blood and relevant organ material for further laboratory examination for this vesicular disease virus. Today, the first line system for detection of virus in the sample material is real time RT-PCR (RT-qPCR). The aim of this study was to investigate the diagnostic utility of stabilized blood for detection of FMDV RNA in this system.

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**EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), ECDC (European Centre for Disease Prevention and Control) and EMA (European Medicines Agency), 2013. Scientific Opinion on the possible risks posed by the Influenza A(H3N2v) virus for animal health and its potential spread and implications for animal and human health.**
Swine are an important host in influenza virus ecology since they are susceptible to infections with both avian and human influenza A viruses. In 2011 and 2012, clusters of human infection with a swine-origin influenza A(H3N2) variant virus (H3N2v) containing the matrix (M) gene from the 2009 H1N1 pandemic virus were reported in the United States (US). The likelihood of introduction of H3N2v virus into the EU, and subsequent exposure and infection of EU pig herds was assessed. The overall likelihood of a pig holding in the EU being infected by exposure to H3N2v virus through either imported infectious pigs or humans coming from the US was estimated to be low. Efficient separation of imported pigs for 30 days would reduce the likelihood of exposure to a negligible level. The likelihood that H3N2v would spread to other pig holdings was judged to be high, assuming frequent movements of pigs between holdings. Currently, applied real time RT-PCRs can detect all swine influenza A viruses and, combined with gene sequencing, would identify the emergence of H3N2v virus. However, sequencing is not done on a routine basis in EU. Experimental studies in pigs show that the infection is purely of respiratory nature and follows a relatively mild course with fever, coughing and inappetence, similar to that of the endemic swine influenza viruses. Immunity resulting from vaccination with European vaccines may provide some cross-protection against infection with H3N2v virus whereas vaccines based on US swine H3N2 strains would offer superior protection. It is not possible to predict which changes within H3N2v virus might enable it to develop pandemic properties. Hence, it is not possible at present to set up a specific system to monitor such a risk. Nevertheless, it is recommended to reinforce the monitoring of influenza strains circulating in pigs in EU.

**General information**
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Experimental infection of pigs with two East European variants of Type 1 PRRSV

Porcine reproductive and respiratory syndrome viruses (PRRSV) have been divided into Type 1 (European) and Type 2 (North American) viruses. PRRSV are very diverse and Type 1 viruses have even been further divided into subtypes. While Type 1 viruses from Western Europe belong to subtype 1, viruses from Eastern Europe have been divided into at least 3 different subtypes based on the length of ORF7 and in addition, atypical Type 1 viruses do not readily group into the subtype groups. In experimental trials it has been shown that some of these viruses, e.g. strain Lena, are more virulent than the subtype 1 strains.

The aim of this project was to study the infection dynamics and clinical and pathological impact of two east European Type 1 strains. In an experimental trial, infection of pigs with the Russian subtype 2 strain “Ili6” and the Belarusian atypical isolate “Bor59” were compared to an early “Lelystad-like” Danish subtype 1 isolate “18794”. Groups of seven pigs of unique high sanitary status were infected with one of the three PRRSV isolates, and a fourth group served as sham-inoculated controls. The pigs were monitored for 24 days, and nasal swabs and blood samples were taken at 0, 3, 7, 10, 14, 17, 21 and 24 days post infection (dpi).

The pigs infected with the “Bor59” virus developed higher body temperature and more severe clinical symptoms compared to the other three groups, although the clinical signs in general were mild. The acute phase response was measured in serum samples as an objective indicator of infection. Acute phase protein C-reactive protein (CRP) showed an increase in levels in pigs infected with the Eastern European viruses with an earlier rise for Bor59 than for Ili6, both peaking at 10 dpi. In contrast, the CRP level did not increase significantly in neither the subtype 1 virus inoculated pigs nor the sham-inoculated controls. Acute phase protein haptoglobin showed a very early increase in Bor59 infected pigs, peaking at 3 dpi, while no increase was observed in Ili6 infected pigs.

All of the virus inoculated pigs seroconverted, as measured by IPMA and ELISA, around 7 dpi, and virus was detected by real-time RT-PCR in serum at various quantities and times after infection; detailed PCR analyses are ongoing.

Taken together, these preliminary data suggested that the east European subtype 2 isolate Ili6 and the atypical Bor59 strain induced more severe infection compared to the type 1 “Lelystad-like” virus isolate. This correlates with results obtained from studies of other east European PRRSV strains.
Genetic and biological characterisation of an avian-like H1N2 swine influenza virus generated by reassortment of circulating avian-like H1N1 and H3N2 subtypes in Denmark

BACKGROUND: The influenza A virus subtypes H1N1, H1N2 and H3N2 are the most prevalent subtypes in swine. In 2003, a reassorted H1N2 swine influenza virus (SIV) subtype appeared and became prevalent in Denmark. In the present study, the reassortant H1N2 subtype was characterised genetically and the infection dynamics compared to an “avian-like” H1N1 virus by an experimental infection study.

METHODS: Sequence analyses were performed of the H1N2 virus. Two groups of pigs were inoculated with the reassortant H1N2 virus and an “avian-like” H1N1 virus, respectively, followed by inoculation with the opposite subtype four weeks later. Measurements of HI antibodies and acute phase proteins were performed. Nasal virus excretion and virus load in lungs were determined by real-time RT-PCR.

RESULTS: The phylogenetic analysis revealed that the reassorted H1N2 virus contained a European “avian-like” H1-gene and a European “swine-like” N2-gene, thus being genetically distinct from most H1N2 viruses circulating in Europe, but similar to viruses reported in 2009/2010 in Sweden and Italy. Sequence analyses of the internal genes revealed that the reassortment probably arose between circulating Danish “avian-like” H1N1 and H3N2 SIVs. Infected pigs developed cross-reactive antibodies, and increased levels of acute phase proteins after inoculations. Pigs inoculated with H1N2 exhibited nasal virus excretion for seven days, peaking day 1 after inoculation two days earlier than H1N1 infected pigs and at a six times higher level. The difference, however, was not statistically significant. Pigs euthanized on day 4 after inoculation, had a high virus load in all lung lobes. After the second inoculation, the nasal virus excretion was minimal. There were no clinical sign except elevated body temperature under the experimental conditions.

CONCLUSIONS: The “avian-like” H1N2 subtype, which has been established in the Danish pig population at least since 2003, is a reassortant between circulating swine “avian-like” H1N1 and H3N2. The Danish H1N2 has an “avian-like” H1 and differs from most other reported H1N2 viruses in Europe and North America/Asia, which have H1-genes of human or “classical-swine” origin, respectively. The variant seems, however, also to be circulating in countries like Sweden and Italy. The infection dynamics of the reassorted “avian-like” H1N2 is similar to the older “avian-like” H1N1 subtype.
Occurrence of Schmallenberg virus in Danish biting midges (Culicoides spp.)

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Processing of the VP1/2A Junction Is Not Necessary for Production of Foot-and-Mouth Disease Virus Empty Capsids and Infectious Viruses: Characterization of “Self-Tagged” Particles

The foot-and-mouth disease virus (FMDV) capsid protein precursor, P1-2A, is cleaved by 3Cpro to generate VP0, VP3, VP1, and the peptide 2A. The capsid proteins self-assemble into empty capsid particles or viruses which do not contain 2A. In a cell culture-adapted strain of FMDV (O1 Manisa [Lindholm]), three different amino acid substitutions (E83K, S134C, and K210E) were identified within the VP1 region of the P1-2A precursor compared to the field strain (wild type [wt]). Expression of the O1 Manisa P1-2A (wt or with the S134C substitution in VP1) plus 3Cpro, using a transient expression system, resulted in efficient capsid protein production and self-assembly of empty capsid particles. Removal of the 2A peptide from the capsid protein precursor had no effect on capsid protein processing or particle assembly. However, modification of E83K alone abrogated particle assembly with no apparent effect on protein processing. Interestingly, the K210E substitution, close to the VP1/2A junction, completely blocked processing by 3Cpro at this cleavage site, but efficient assembly of “self-tagged” empty capsid particles, containing the uncleaved VP1-2A, was observed. These self-tagged particles behaved like the unmodified empty capsids in antigen enzyme-linked immunosorbent assays and integrin receptor binding assays. Furthermore, mutant viruses with uncleaved VP1-2A could be rescued in cells from full-length FMDV RNA transcripts encoding the K210E substitution in VP1. Thus, cleavage of the VP1/2A junction is not essential for virus viability. The production of such engineered self-tagged empty capsid particles may facilitate their purification for use as diagnostic reagents and vaccines.

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Schmallenberg virus experimental infection of sheep

Since late 2011, a novel orthobunyavirus, named Schmallenberg virus (SBV), has been implicated in many cases of severely malformed bovine and ovine offspring in Europe. In adult cattle, SBV is known to cause a mild transient disease; clinical signs include short febrile episodes, decreased milk production and diarrhoea for a few days. However, the knowledge about clinical signs and pathogenesis in adult sheep is limited. In the present study, adult sheep of European domestic breeds were inoculated with SBV either as cell culture grown virus or as virus with no history of passage in cell cultures. Various experimental set-ups were used. Sampling included blood collection at different time points during the experimental period and selected organ material at autopsy. Data from this study showed, that the RNAemic period in sheep was as short as reported for cattle; viral genome was detectable for about 3–5 days by real-time RT-PCR. In total, 13 out of 30 inoculated sheep became RNAemic, with the highest viral load in animals inoculated with virus from low cell culture passaged or the animal passaged material. Contact animals remained negative throughout the study. One RNAemic sheep showed diarrhoea for several days, but fever was not recorded in any of the animals. Antibodies were first detectable 10–14 days post inoculation. Viral RNA was detectable in spleen and lymph nodes up to day 44 post inoculation. In conclusion, as described for cattle, SBV-infection in adult sheep predominantly results in subclinical infection, transient RNAemia and a specific antibody response. Maintenance of viral RNA in the lymphoreticular system is observed for an extended period.
Transplacental transmission of field and rescued strains of BTV-2 and BTV-8 in experimentally infected sheep

Transplacental transmission of bluetongue virus has been shown previously for the North European strain of serotype 8 (BTV-8) and for tissue culture or chicken egg-adapted vaccine strains but not for field strains of other serotypes. In this study, pregnant ewes (6 per group) were inoculated with either field or rescued strains of BTV-2 and BTV-8 in order to determine the ability of these viruses to cross the placental barrier. The field BTV-2 and BTV-8 strains was passaged once in Culicoides KC cells and once in mammalian cells. All virus inoculated sheep became infected and seroconverted against the different BTV strains used in this study. BTV RNA was detectable in the blood of all but two ewes for over 28 days but infectious virus could only be detected in the blood for a much shorter period. Interestingly, transplacental transmission of BTV-2 (both field and rescued strains) was demonstrated at high efficiency (6 out of 13 lambs born to BTV-2 infected ewes) while only 1 lamb of 12 born to BTV-8 infected ewes showed evidence of in utero infection. In addition, evidence for horizontal transmission of BTV-2 between ewes was observed. As expected, the parental BTV-2 and BTV-8 viruses and the viruses rescued by reverse genetics showed very similar properties to each other. This study showed, for the first time, that transplacental transmission of BTV-2, which had been minimally passaged in cell culture, can occur; hence such transmission might be more frequent than previously thought.

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Research output: Contribution to journal › Journal article – Annual report year: 2013 › Research › peer-review
Analysis of viremia and transplacental transmission of field and rescued strains of BTV-2 and BTV-8 following inoculation of pregnant sheep

Objectives
Live bluetongue virus (BTV) vaccine-strains and also, surprisingly, the European strain of BTV-8 can cross the placental barrier and thus pass from one generation of animals to another without involvement of the insect vector. A better understanding of the genetic basis for the transmission characteristics of the virus would help to identify the risks posed by further BTV incursions and facilitate the design of better control strategies. The development of reverse genetics for BTV enables investigation of the genetic traits conferred by individual genome segments within rescued viruses by making defined reassortants. To date, only a few experiments have investigated whether field and rescued virus strains behave similarly in vivo.

Methods
Twenty-four sheep (in 4 groups of 6) were inoculated (s.c.) with 4 strains of BTV in late pregnancy (approx. 1 month before lambing). The viruses used were: BTV-2 wt (Italian field strain), BTV-2 (rescued), BTV-8 wt (field strain from the Netherlands) and BTV-8 (rescued). Four sheep were non-inoculated controls. Blood samples from the sheep were tested frequently for viremia and anti-BTV antibodies (by ELISA) in the period until lambing. Pre-colostral blood samples were collected from all newborn lambs, except for one born dead, to determine if transplacental transmission had occurred. Milk from ewes was collected daily for 7 days after lambing and blood samples from the lambs were collected on days 0, 3 and 7 after birth. All samples have being tested for the presence of anti-BTV antibodies and for virus (RT-qPCR).

Results
All inoculated animals developed viremia. The viremia was significantly higher at all sampling points following inoculation (p<0.01 or p< 0.05, Mann-Whitney's U Test) in animals inoculated with BTV-2 wt compared to animals inoculated with BTV-2 rescued, whereas no significant difference was detected between BTV-8 wt and BTV-8 rescued. Wild type virus infected animals had a longer lag phase before antibodies were detected but the response increased at a faster rate. Some of the animals displayed clinical signs of infection, e.g. fever and panting. All the ewes delivered one lamb each, a few lambs born early did not thrive and were euthanized but most appeared healthy. Seven of the 28 lambs had been infected transplacentally; 2 from ewes inoculated with BTV-2 wt, 3 from ewes inoculated with BTV-2 rescued and 1 from a ewe inoculated with BTV-8 wt. The last infected lamb was from a non-inoculated control sheep, in the same stable but physically separated from, the BTV-2 wt inoculated ewes and became viremic with BTV-2 10 days after the others were inoculated.

Conclusion
Both wild-type and rescued BTVs induced viremia. Surprisingly, transplacental transmission occurred more frequently in ewes inoculated with BTV-2, both wt and rescued, than in ewes inoculated with BTV-8. The BTV-2 wt was passaged once in Kc and once in CPT-Tert cells. These very few passages may be enough to introduce changes enabling the virus to cross the placental barrier. This experiment indicates it will be difficult to identify a single BTV segment responsible for transplacental transmission in sheep using rescued BTV-2 and BTV-8 strains.

Capsid coding sequences of foot-and-mouth disease viruses are determinants of pathogenicity in pigs
The surface exposed capsid proteins, VP1, VP2 and VP3, of foot-and-mouth disease virus (FMDV) determine its antigenicity and the ability of the virus to interact with host-cell receptors. Hence, modification of these structural proteins may alter the properties of the virus. In the present study we compared the pathogenicity of different FMDVs in young pigs. In total 32 pigs, 7-weeks-old, were exposed to virus, either by direct inoculation or through contact with inoculated pigs, using cell culture adapted (O1K B64), chimeric (O1K/A-TUR and O1K/O-UKG) or field strain (O-UKG/34/2001) viruses. The O1K B64 virus and the two chimeric viruses are identical to each other except for the capsid coding region. Animals exposed to O1K B64 did not exhibit signs of disease, while pigs exposed to each of the other viruses showed typical clinical signs of foot-and-mouth disease (FMD). All pigs infected with the O1K/O-UKG chimera or the field strain (O-
UKG/34/2001 developed fulminant disease. Furthermore, 3 of 4 in-contact pigs exposed to the O1K/O-UKG virus died in the acute phase of infection, likely from myocardial infection. However, in the group exposed to the O1K/A-TUR chimeric virus, only 1 pig showed symptoms of disease within the time frame of the experiment (10 days). All pigs that developed clinical disease showed a high level of viral RNA in serum and infected pigs that survived the acute phase of infection developed a serotype specific antibody response. It is concluded that the capsid coding sequences are determinants of FMDV pathogenicity in pigs.

Comparison of the pathogenicity of two serotype O foot-and-mouth disease viruses (chimeric and field strain viruses) in pigs

Culicoids as Vectors of Schmallenberg Virus
Evidence for Culicoides obsoletus group as vector for Schmallenberg virus in Denmark

Schmallenberg virus (SBV) was first identified in Germany in late 2011 by the Friedrich Loeffler Institute and has now been found in several European countries including Holland, France, Belgium, U.K. and Spain. The disease, which affects sheep, cattle and goats, was first recognized due to transient clinical symptoms including fever, diarrhea and loss of milk production. However, a more significant consequence of infection in pregnant animals is the production of severe congenital malformations in newborn animals, especially lambs. The virus is a member of the Orthobunyavirus genus.
within the Bunyaviridae family and is closely related to Shamonda and Akabane viruses. These viruses are transmitted by insect vectors (including biting midges (Culicoides sp.) and mosquitoes). To determine whether these insects may act as vectors for SBV, biting midges (Culicoides spp.) caught in October 2011, in the south-west of Denmark (close to the German border), were sorted into pools and tested for the presence of Schmallenberg virus RNA by RT-qPCR. From 18 pools of 5 midges from the C. obsoletus group, 2 pools were both found positive in two separate assays, targeting the L- and S- segments of the SBV RNA. However, 4 pools of C. punctatus s.str were negative. The sequence of 80bp (excluding the primer sequences) from the amplicons (ca. 145bp) was identical to that published for the expected region of the SBV L-segment. The levels of SBV RNA detected in the biting midges were much higher than could be accounted for due to the residue of a blood meal and no ruminant actin mRNA could be detected either. These results strongly suggest that SBV has replicated within specimens of the C. obsoletus group and indicates that these biting midges can act as vectors for this virus. To date (end of March), no cases of disease due to SBV have been detected in sheep, cattle or goats in Denmark.

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Mund- og klovesyge - et fælles globalt problem

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"Schmallenberg" virus: Analysis of the Epidemiological Data and Assessment of Impact: EFSA-Q-2012-00305
This scientific report provides an overall assessment of the impact of the infection on animal health, animal production and animal welfare of the provisionally named "Schmallenberg" virus (SBV) first detected in Germany. In Europe, 3745 holdings have been reported with SBV cases confirmed by laboratory testing across several Member States, mid May 2012. EFSA reviewed the epidemiological reports noting that SBV has been detected in cattle, sheep, goats and a bison. SBV antibodies have been detected in deer and no other species are known to be affected. EFSA also confirms that new studies support the initial assessment undertaken by the European Center for Disease Control and Prevention, that it is
very unlikely that SBV poses a risk to humans. In terms of transmission routes, recent entomological investigations have identified SBV in field samples of biting midges of the Culicoides obsoletus group. Currently there is no evidence of any other route of transmission other than transplacental or vector borne routes. EFSA coordinated the collation of SBV epidemiological data during 2011-2012 in order to obtain comparable data for Europe. The maximum proportion of reported sheep holdings with SBV confirmed was 4% per country and 7.6% per region while for cattle less than 1.3% of holdings were reported as SBV confirmed at both country and regional level. In order to assess the impact of SBV(spatial and temporal spread, proportion of affected holding and potential projection of arthrogryposis hydranencephaly syndrome cases) three models were used. In regions with SBV confirmed holdings, assuming a high prevalence of infection and post infection immunity, impact in the 2012-2013 calving and lambing season should be low. However, assuming SBV survived the winter of 2011, the models suggest that in unaffected regions with suitable temperatures for within herd transmission by vectors and high density of susceptible species (cattle and sheep) SBV infection is likely to spread. EFSA puts forward a number of recommendations to fill the knowledge gaps, these include but are not limited to: continuing serological investigations in affected regions and regions neighbouring affected areas, within herd and animal level impact investigation, monitoring putative vector population, setting SBV host vector transmission parameters, investigating other routes of transmission, host susceptibility, virulence and vulnerable period during gestation. Furthermore, the possible origins of the virus should be investigated as more information becomes available on the virus characteristics and infection epidemiology.

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Schmallenberg virus fundet i mitter i Danmark

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Contributors: Rasmussen, L. D., Kristensen, B., Kirkeby, C., Rasmussen, T. B., Belsham, G., Bødker, R., Bøtner, A.
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ISI indexed (2012): ISI indexed no
Original language: Danish
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Source: dtu
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Research output: Contribution to journal › Journal article – Annual report year: 2012 › Research › peer-review
Virus survival in slurry: Analysis of the stability of foot-and-mouth disease, classical swine fever, bovine viral diarrhoea and swine influenza viruses

Farm slurry can be highly contaminated with viral pathogens. The survival of these pathogens within slurry is important since this material is often distributed onto farm land either directly or after heat treatment. There is clearly some risk of spreading pathogens in the early stages of an outbreak of disease before it has been recognized. The survival of foot-and-mouth disease virus, classical swine fever virus, bovine viral diarrhoea virus and swine influenza virus, which belong to three different RNA virus families plus porcine parvovirus (a DNA virus) was examined under controlled conditions. For each RNA virus, the virus survival in farm slurry under anaerobic conditions was short (generally ≤1h) when heated (to 55°C) but each of these viruses could retain infectivity at cool temperatures (5°C) for many weeks. The porcine parvovirus survived considerably longer than each of the RNA viruses under all conditions tested. The implications for disease spread are discussed.

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Contributors: Bøtner, A., Belsham, G.
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Capsid proteins from field strains of foot-and-mouth disease virus confer a pathogenic phenotype in cattle on an attenuated, cell-culture-adapted virus

Chimeric foot-and-mouth disease viruses (FMDVs) have been generated from plasmids containing full-length FMDV cDNAs and characterized. The parental virus cDNA was derived from the cell-culture-adapted O1Kaufbeuren B64 (O1K B64) strain. Chimeric viruses, containing capsid coding sequences derived from the O/UKG/34/2001 or A/Turkey 2/2006 field viruses, were constructed using the backbone from the O1K B64 cDNA, and viable viruses (O1K/O-UKG and O1K/A-Tur, respectively) were successfully rescued in each case. These viruses grew well in primary bovine thyroid cells but grew less efficiently in BHK cells than the rescued parental O1K B64 virus. The two chimeric viruses displayed the expected antigenicity in serotype-specific antigen ELISAs. Following inoculation of each virus into cattle, the rescued O1K B64 strain proved to be attenuated whereas, with each chimeric virus, typical clinical signs of foot-and-mouth disease were observed, which then spread to in-contact animals. Thus, the surface-exposed capsid proteins of the O1K B64 strain are responsible for its attenuation in cattle. Consequently, there is no evidence for any adaptation, acquired during cell culture, outside the capsid coding region within the O1K B64 strain that inhibits replication in cattle. These chimeric infectious cDNA plasmids provide a basis for the analysis of FMDV pathogenicity and characterization of receptor utilization in vivo.

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Contributors: Bøtner, A., Kakker, N. K., Barbezange, C., Berryman, S., Jackson, T., Belsham, G.
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Publication date: 2011
Peer-reviewed: Yes
EFSA Panel on Animal Health and Welfare (AHAW); Scientific Opinion on bluetongue monitoring and surveillance: EFSA-Q-2010-01238

Following a request from the Commission, the Panel on Animal Health and Welfare was asked to deliver a Scientific Opinion on: 1) the expected prevalence (design prevalence) under different circumstances, and, 2) an updated scientific assessment of the size of the relevant geographical area for the purpose of monitoring and surveillance programmes for bluetongue. A systematic literature review and a review of monitoring and surveillance data from European Union Member States was performed in order to estimate the prevalences observed in the Member States. The prevalences observed in areas that have been infected for several years were slightly lower than the design prevalence of 2% currently used for monthly testing of sentinel animals, but much lower than the design prevalences of 20% and 10% for annual surveys in populations of unvaccinated and vaccinated ruminants, respectively. Currently there is no scientific evidence that suggests an optimal size of the relevant geographic unit for BTV monitoring and surveillance, since it depends on many factors, including the goal of the surveillance programmes. Early warning based on passive surveillance will take place irrespective of the size of the geographical unit but, when based on active surveillance, it is best targeted at regions considered at risk for introduction, using small geographical units, a high sampling frequency and sample size. For estimating the impact of interventions on the prevalence of infected animals, smaller areas result in more precise estimates of the prevalence and also take better account of local differences. For establishing freedom from infection, smaller areas result in lower design prevalence for a region as a whole and take better account of local differences in infection dynamics.

EFSA Panel on Animal Health and Welfare (AHAW); Scientific Opinion on bluetongue serotype 8: EFSA-Q-2010-01237

To answer a question from the European Commission on the potential special characteristics of bluetongue virus (BTV) serotype 8 (BTV-8) compared to other serotypes and their possible impact on the epidemiology of the disease, a systematic literature review was carried out by a working group established by the Animal Health and Welfare Panel. Currently, three special features can be assigned to BTV-8, which are the ability to cause serious disease in cattle and...
goats, the ability to be transmitted transplacentally, and the ability to contaminate semen. The transplacental transmission and the contamination of semen are also observed for several serotypes of modified live virus (MLV) vaccines and for some cell culture/egg passaged strains. These two features may have an impact on the epidemiology of the disease, since they may increase the ability of BTV-8 to survive the winter period, for example, when pregnant cows are infected in late autumn and give birth to viraemic offspring in the next vector season, or, through infecting the recipient dam via artificial insemination (AI) with frozen contaminated semen. Furthermore, the chance of BTV-8 spread may be increased either through movement of seropositive but virus negative pregnant animals, which may give birth to viraemic calves, or through natural mating or AI using BTV-8 contaminated semen by transmission from semen to receiving dam. The current legislation provides effective measures to ensure that all dams are immune to BTV before insemination or mating, so there is no subsequent risk of transplacental infection of their offspring. Furthermore, pregnant animals are effectively restricted in their movement. More research is needed to determine whether oral transmission and/or transmission through embryo transfer are more likely to occur for BTV-8 than for other BTV serotypes.

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Following the emergence in 2009 of the new pandemic H1N1 influenza virus, which contained gene segments from pig, bird and human influenza viruses, it was apparent that a better scientific understanding is required of influenza viruses to protect public and animal health. The latest scientific data on biological properties of the virus, transmissibility, host susceptibility and epidemiology has been evaluated in order to identify factors that could be monitored in animals and that would suggest a risk of emergence of a new pandemic influenza strains. Virological studies and animal models have highlighted the importance of individual virus proteins but virulence and transmissibility are polygenic effects and no single genetic marker can be reliably associated with increased pathogenicity or transmissibility. It was concluded that current monitoring of the influenza gene pool in humans has been able to provide an alert for the emergence of new human influenza strains of public health significance. In contrast, there is an incomplete view of the influenza virus strains circulating among pigs and birds at the global level. Interpretation of the origins and pandemic potential of influenza viruses do require knowledge of the influenza gene pools in both pigs and birds, as well as other animal species. It is recommended that there should be long term support for a passive monitoring network in pigs and birds in order to promote greater understanding of the evolution of influenza viruses at the global level. Maximum benefit can only be obtained by applying an integrated approach involving the medical and veterinary networks including development of harmonised tools and approaches, exchange of virus strains and sequence data and enhancing the coordination and dissemination of the findings from the human, swine and avian networks.

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Original language: English
Rescue of foot-and-mouth disease viruses that are pathogenic for cattle from preserved viral RNA samples

Background: Foot and mouth disease is an economically important disease of cloven-hoofed animals including cattle, sheep and pigs. It is caused by a picornavirus, foot-and-mouth disease virus (FMDV), which has a positive sense RNA genome which, when introduced into cells, can initiate virus replication. Principal Findings: A system has been developed to rescue infectious FMDV from RNA preparations generated from clinical samples obtained under experimental conditions and then applied to samples collected in the "field". Clinical samples from suspect cases of foot-and-mouth disease (FMD) were obtained from within Pakistan and Afghanistan. The samples were treated to preserve the RNA and then transported to National Veterinary Institute, Lindholm, Denmark. Following RNA extraction, FMDV RNA was quantified by real-time RT-PCR and samples containing significant levels of FMDV RNA were introduced into susceptible cells using electroporation. Progeny viruses were amplified in primary bovine thyroid cells and characterized using antigen ELISA and also by RT-PCR plus sequencing. FMD viruses of three different serotypes and multiple lineages have been successfully rescued from the RNA samples. Two of the rescued viruses (of serotype O and Asia 1) were inoculated into bull calves under high containment conditions. Acute clinical disease was observed in each case which spread rapidly from the inoculated calves to in-contact animals. Thus the rescued viruses were highly pathogenic. The availability of the rescued viruses enabled serotyping by antigen ELISA and facilitated genome sequencing. Conclusions: The procedure described here should improve the characterization of FMDVs circulating in countries where the disease is endemic and thus enhance disease control globally.

Screening of reservoirs for hepatitis E virus

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Contributors: Belsham, G., Jamal, S. M., Tjørnehøj, K., Bøtner, A.
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Research output: Contribution to journal › Journal article – Annual report year: 2011 › Research › peer-review
Detection of myxoma viruses encoding a defective M135R gene from clinical cases of myxomatosis; possible implications for the role of the M135R protein as a virulence factor

Background: Myxoma virus is a member of the Poxviridae and causes disease in European rabbits. Laboratory confirmation of the clinical disease, which occurs in the autumn of most years in Denmark, has been achieved previously using antigen ELISA and electron microscopy. Results: An unusually large number of clinically suspected cases of myxomatosis were observed in Denmark during 2007. Myxoma virus DNA was detected, using a new real time PCR assay which targets the M029L gene, in over 70% of the clinical samples submitted for laboratory confirmation. Unexpectedly, further analysis revealed that a high proportion of these viral DNA preparations contained a frame-shift mutation within the M135R gene that has previously been identified as a virulence factor. This frame-shift mutation results in expression of a greatly truncated product. The same frame-shift mutation has also been found recently within an avirulent strain of myxoma virus (6918). However, three other frame-shift mutations found in this strain (in the genes M009L, M036L and M148R) were not shared with the Danish viruses but a single nucleotide deletion in the M138R/M139R intergenic region was a common feature. Conclusions: It appears that expression of the full-length myxoma virus M135R protein is not required for virulence in rabbits. Hence, the frame-shift mutation in the M135R gene in the nonpathogenic 6918 virus strain is not sufficient to explain the attenuation of this myxoma virus but one/some of the other frame-shift mutations alone or in conjunction with one/some of the thirty two amino acid substitutions must also contribute. The real time PCR assay for myxoma virus is a useful diagnostic tool for laboratory confirmation of suspected cases of myxomatosis.
EFSA Panel Animal Health and Welfare (AHAW); Scientific Opinion on the pandemic (H1N1) 2009 influenza and its potential implications for animal health. EFSA-Q-2009-00935

Analysis of the recent pandemic (H1N1) 2009 (pH1N1) virus indicates a probable origin in pigs. However, it was not reported in pigs prior to its detection in humans. Several cases of pH1N1 virus infections in animals have been reported, mainly in pigs but also in other animals including turkeys. Occasionally, pigs have been infected following exposure to pH1N1 infected humans. In pigs, a subclinical course was common and when clinical signs were seen (coughing, fever) they were generally mild. Presently, the clinical impact of pH1N1 virus on the EU pig population is considered minimal. In poultry, outbreaks of pH1N1 have been reported only in turkey breeder flocks. So far, there is no evidence that pH1N1 virus is able to spread horizontally among turkeys. Awareness should be raised about the risk of infecting breeder turkeys with pH1N1 virus during artificial insemination. To date, no infection of wild birds with pH1N1 virus has been reported. From an animal health perspective, no specific disease control measures are considered necessary. Vaccines based on the pH1N1 virus appear to induce protection in swine similar to that induced by the existing swine influenza virus (SIV) vaccines. Such vaccines efficiently prevent disease by reducing virus replication in the lungs. However, voluntary vaccination of swine with these vaccines has not halted the circulation of SIV in swine. There is no urgency for vaccination of pigs against pH1N1 virus. Currently, no vaccines against H1 viruses for poultry are available but at present, there is no need to vaccinate poultry against pH1N1 virus. Monitoring of circulating influenza viruses in swine and poultry populations should be instigated to monitor the evolution of the pH1N1 virus including changes in virulence.

The risk that African Swine Fever virus (ASFV) remains endemic in the Trans Caucasian Countries (TCC) and the Russian Federation (RF) is moderate, while the risk of its spread in these regions is high. The resulting risk of introduction from these regions into the EU is moderate most likely through food waste. The risk of ASFV remaining endemic in wild boar and the consequent introduction into the EU was considered low in the TCC and moderate in the RF, mainly due to the higher population density in the RF and the connected wild boar populations to the EU from the RF. Within the EU, mainly domestic pigs in the free range (FR) and the limited biosecurity sector (LB) are likely to be exposed to ASFV via swill feeding, with low risk. Once infected, the risk of spread from the LB and FR sectors prior detection is high, mainly due to movement of pigs, people and vehicles and moderate from the High Biosecurity (HB) sector. The risk of endemicity in domestic pigs is considered negligible in HB and low in LB since the implementation of control measures are effective. The risk of endemicity in the FR sector is moderate due to wild boar contact, non-compliance with animal movement ban and difficult access to all individual pigs. The risk of ASFV becoming endemic in the wild boar population in the EU is moderate, in particular in areas with connected wild boar populations. Because of their long life, ticks of the O. erraticus complex can be important in maintaining local foci of ASFV, where pigs are kept under traditional systems. Ticks do not, play an active role in the geographical spread of the virus. Wild boar have never been found infested because they do not rest inside burrows potentially infested by ticks.

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Bluetongue i Danmark 2008

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Induction of porcine post-weaning multisystemic wasting syndrome (PMWS) in pigs from PMWS unaffected herds following mingling with pigs from PMWS-affected herds

In this paper we present the results from two experimental studies (I and II) investigating whether post-weaning multisystemic wasting syndrome (PMWS) can be induced in pigs from PMWS unaffected herds by mingling with pigs from PMWS-affected herds and to observe whether transportation and/or mingling of healthy pigs from unaffected herds could induce PMWS. The studies comprised pigs from 12 different herds. Eight herds had PMWS while four were unaffected. All 12 herds were found to be infected with PCV2. Pigs from PMWS-affected herds were mingled with pigs from unaffected herds in four separate compartments in both study I and study II. In addition, in study II, four groups of pigs from unaffected herds were included. Two groups with pigs transported and mingled from unaffected herds and two groups with pigs which were only transported. The PMWS diagnoses on the individual pigs were based on lymphoid depletion, histiocytic proliferation and the presence of giant cells or inclusion bodies together with the demonstration of PCV2 in lymphoid tissue. Healthy pigs, in both studies, developed PMWS 4–5 weeks after mingling with pigs clinically affected with PMWS. None of the pigs from unaffected herds which had no contact with pigs from PMWS-affected herds developed clinical signs of PMWS. Transportation and mingling of pigs from PMWS unaffected herds in combination or alone was insufficient to provoke PMWS.
Infection, excretion and seroconversion dynamics of porcine circovirus type 2 (PCV2) in pigs from post-weaning multisystemic wasting syndrome (PMWS) affected farms in Spain and Denmark

Longitudinal case-control studies were performed in post-weaning multisystemic wasting syndrome (PMWS) affected farms from Denmark and Spain using similar designs. Fourteen independent batches of 100-154 pigs per batch were monitored from birth to PMWS outbreak occurrence. Pigs displaying PMWS-like signs and matched healthy cohorts were euthanized during the clinical outbreak. PMWS was diagnosed according to internationally accepted criteria and pigs were classified as: (i) PMWS cases, (ii) wasted non-PMWS cases and (iii) healthy pigs. Porcine circovirus type 2 (PCV2) quantitative PCR (qPCR) and serology techniques were applied to analyse longitudinally collected sera and/or nasal and rectal swabs. Results showed that PCV2 load increased in parallel to waning maternal antibody levels, reaching the maximum viral load concurrent with development of clinical signs. PMWS affected pigs had higher PCV2 prevalence and/or viral load than healthy pigs in all collected samples at necropsy (p <0.0001-0.05) and even in sera and nasal swabs at the sampling prior to PMWS outbreak (p <0.01-0.05). Danish farms had a higher PCV2 prevalence in young piglets as well as an earlier PMWS presentation compared to Spanish farms. PMWS diagnoses were confirmed by laboratory tests in only half of pigs clinically suspected to suffer from PMWS. Positive and significant correlations were found among PCV2 viral loads present in sera, nasal swabs, rectal swabs and lymphoid tissues (R = 0.289-0.827, p <0.0001-0.01), which indicates that nasal and rectal swabs were suitable indicators of PCV2 excretion. Sensitivity and/or specificity values observed from both tests used separately or combined suggested that qPCR and/or serology tests are not apparently able to substitute histopathology plus detection of PCV2 in tissues for the individual PMWS diagnosis within PMWS affected farms. However, qPCR appears to be a potential reliable technique to diagnose PMWS on a population basis.
Antibody titers against swine influenza subtypes determined by the hemagglutination inhibition test are highly dependent on the strain

In Denmark there are three circulating strains of swine influenza H1N1, H1N2 and H3N2. The H1N2 is different from the H1N2 subtypes circulating in other European countries. The Danish subtype is a reassortment between the two Danish circulating swine influenza subtypes H1N1 and H3N2. From a diagnostic and epidemiological point of view it is crucial to clarify whether the immunological response to one subtype protects against infection with the other subtype. The hemagglutination inhibition test (HI-test) has been used widely to determine the presence of antibodies in serum against influenza viruses. In the present study the HI-test was used to determine antibody response from experimental infected pigs. The aim of the study was to evaluate the antibody response against the new Danish influenza subtype H1N2 (H1N2dk) and to examine the level of crossprotection/reaction between the two influenza subtypes.

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Contributors: Trebbien, R., Nielsen, J., Bøtner, A., Hjulsager, C. K., Larsen, L. E.
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Dynamics of porcine circovirus type 2 infection and excretion in pigs from postweaning multisystemic wasting syndrome affected farms from Spain and Denmark
Serological and non-quantitative DNA detection techniques (PCR) have been widely used to monitor porcine circovirus type 2 (PCV2) infection dynamics (1,2). In spite of available epidemiological information, very few data on PCV2 load dynamics of Postweaning multisystemic wasting syndrome (PMWS) affected and non-affected pigs in PMWS affected farms are available. The present longitudinal study describes the evolution of PCV2 infection and excretion in pathologically characterized pigs from PMWS affected farms from two different countries, namely Denmark and Spain.

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Examination for a viral co-factor in postweaning multisystemic wasting syndrome (PMWS)
In order to test the hypothesis that a putative co-factor for the development of postweaning multisystemic wasting syndrome (PMWS) in pigs could be of viral origin, we performed extensive virological examinations on organ material from pigs diagnosed with PMWS originating from within a Danish PMWS-transmission study. Virus isolation attempts were carried out on a large panel of different cell types including primary pig kidney cells and lung macrophages, primary rabbit kidney cells and seven established cell lines (MARC-145, ST117, PK15, BHK21, HeLa, Vero, and MDCK). Although these represent cells with susceptibility to a wide range of known viruses, the results did not provide evidence for a specific virus other than PCV2 contributing to the development of PMWS. Furthermore, in order to test whether specific genotypes of PCV2 may trigger the switch from PCV2 infection to clinical disease, we compared complete DNA genome sequences of PCV2 derived from PMWS-positive as well as PMWS-negative pigs. On the basis of the DNA sequences, the PCV2 isolates were divided into two groups. Group 1 consisting of one isolate originating from a herd unaffected by PMWS, with group 2 consisting of nine isolates originating from four PMWS-affected herds, four PMWS-positive pigs plus one unaffected herd. The PCV2 genomes from the two groups showed 95.5% identity. Alignment analyses of the sequences encoding the replicase and capsid protein from group 1 and group 2 PCV2 isolates showed two amino acid differences encoded in the replicase protein, while 19 amino acid differences were predicted among the capsid protein sequences. The PCV2 DNA sequence analysis supports recent observations from studies in USA as well as Europe, which suggest
that strain variations may influence the clinical outcome of PCV2 infection.

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Postweaning multisystematic wasting syndrome in Danish pig herds: productivity, clinical signs and pathology

A case-control study of 74 herds with postweaning multisystemic wasting syndrome (PMWS) and 74 matched control herds was carried out. In the case herds the mortality rates of weaner and finisher pigs were 11.2 and 5.2 per cent respectively, compared with 3.1 and 3.2 per cent in the control herds. In most case herds, PMWS developed within the first four weeks after weaning. Wasting, diarrhoea and respiratory signs were observed in 10 per cent of the weaner pigs (7 to 30 kg) in the case herds compared with 7 per cent in the control herds. The average daily gains of the weaner pigs and finisher pigs were 36 g and 52 g less in the case herds than in the control herds. By examining three weaner pigs from each herd the PMWS diagnosis was confirmed by histopathology and immunohistochemistry in 78 per cent of the case herds, but at least one PMWS-positive weaner pig was found in 19 of the control herds. The prevalence of PMWS-positive pigs among illthriven weaner pigs was 45 per cent (101/222) in the case herds, and 12 per cent (27/222) in the control herds. Specific gross pathological findings were associated with a positive PMWS diagnosis; pigs with heavy, rubber-like lungs, atonic intestines, and enlarged bronchial and inguinal lymph nodes, had a 0.7 probability of a positive PMWS diagnosis by laboratory examinations. However, for illthriven pigs, this probability of having PMWS was equal in the case herds and the control herds.
Post-weaning multisystemic wasting syndrome (PMWS) in Danish pig herds: productivity, clinical symptoms, and pathology

A case-control study of 74 herds with postweaning multisystemic wasting syndrome (PMWS) and 74 matched control herds was carried out. In the case herds the mortality rates of weaner and finisher pigs were 11·2 and 5·2 per cent respectively, compared with 3·1 and 3·2 per cent in the control herds. In most case herds, PMWS developed within the first four weeks after weaning. Wasting, diarrhoea and respiratory signs were observed in 10 per cent of the weaner pigs (7 to 30 kg) in the case herds compared with 7 per cent in the control herds. The average daily gains of the weaner pigs and finisher pigs were 36 g and 52 g less in the case herds than in the control herds. By examining three weaner pigs from each herd the PMWS diagnosis was confirmed by histopathology and immunohistochemistry in 78 per cent of the case herds, but at least one PMWS-positive weaner pig was found in 19 of the control herds. The prevalence of PMWS-positive pigs among ill-thriven weaner pigs was 45 per cent (101/222) in the case herds, and 12 per cent (27/222) in the control herds. Specific gross pathological findings were associated with a positive PMWS diagnosis; pigs with heavy, rubber-like lungs, atonic intestines, and enlarged bronchial and inguinal lymph nodes, had a 0·7 probability of a positive PMWS diagnosis by laboratory examinations. However, for ill-thriven pigs, this probability of having PMWS was equal in the case herds and the control herds.
Rabies 2007

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Christiansen, A., Cowan, S., Bøtner, A.
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Peer-reviewed: Unknown

Publication information
Journal: Epi-Nyt
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Source: orbit
Source-ID: 232991

Bluetongue in Europe with focus on the recent introduction of bluetongue virus in north-western Europe

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A.
Pages: 58-60
Publication date: 2007

Host publication information
Title of host publication: Proceedings of Cattle Consultancy Days
Source: orbit
Source-ID: 241780

Bluetongue: The virus, clinical signs, transmission and diagnosis

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A.
Publication date: 2007
Peer-reviewed: No

Publication information
Journal: KU-Life
Original language: Danish
Source: orbit
Source-ID: 242220

Myxomatose hos kaniner på Sjælland og Lolland

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Hammer, A. S., Bøtner, A.
Publication date: 2007
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Publication information
Journal: DVT
Original language: Danish
**Pathology and diagnosis of PMWS in a Danish case – control study**

**General information**
Publication status: Published
Organisations: Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Division of Microbiology and Risk Assessment, National Food Institute, Sektion for Eksotiske Virussygdomme, Division of Virology, Section for Veterinary Epidemiology and public sector consultancy
Publication date: 2007
Peer-reviewed: Yes
Event: Abstract from 5th International Symposium on Emerging and Re-emerging Pig Diseases, Krakow, Poland.
Source: orbit
Source-ID: 241483
Research output: Contribution to conference › Conference abstract for conference – Annual report year: 2007 › Research peer-review

**Pathology and Diagnosis of PMWS in a Danish Case-Control Study**

**General information**
Publication status: Published
Organisations: Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Division of Microbiology and Risk Assessment, National Food Institute, Sektion for Eksotiske Virussygdomme, Division of Virology, Section for Veterinary Epidemiology and public sector consultancy
Publication date: 2007
Peer-reviewed: No
Event: Poster session presented at 5th International Symposium on Emerging and Re-emerging Pig Diseases, Krakow, Poland.
Source: orbit
Source-ID: 241778
Research output: Contribution to conference › Poster – Annual report year: 2007 › Research

**Absence of PCV2-neutralizing antibodies in PMWS-affected pigs**

**General information**
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Lefebvre, D., Meerts, P., Misinzo, G., Nielsen, J., Bøtner, A., Kristensen, C. S., Nauwynck, H. J.
Number of pages: 175
Publication date: 2006

**Host publication information**
Title of host publication: Proceeding of the 19th International Pig Veterinary Congress
Source: orbit
Source-ID: 241731
Research output: Chapter in Book/Report/Conference proceeding › Article in proceedings – Annual report year: 2006 › Research

**A Danish case-control study on risk factors for PMWS – bio security in the herd**

**General information**
Publication status: Published
Organisations: Section for Veterinary Epidemiology and public sector consultancy, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Sektion for Eksotiske Virussygdomme, Division of Virology, Section for Veterinary Diagnostics
Number of pages: 163
A Danish case-control study on risk factors for PMWS-biosecurity in the herd

General information
Publication status: Published
Organisations: Section for Veterinary Epidemiology and public sector consultancy, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Division of Microbiology and Risk Assessment, National Food Institute, Sektion for Eksotiske Virussygdomme, Division of Virology, Section for Veterinary Diagnostics
Publication date: 2006
Peer-reviewed: Yes
Event: Abstract from 19th International Pig Veterinary Society Congress, Copenhagen, Denmark.
Source: orbit
Source-ID: 241478
Research output: Contribution to conference › Conference abstract for conference – Annual report year: 2006 › Research › peer-review

Association between PMWS and PRRSV

General information
Publication status: Published
Organisations: National Veterinary Institute, Section for Veterinary Epidemiology and public sector consultancy, Division of Veterinary Diagnostics and Research, Sektion for Eksotiske Virussygdomme, Division of Virology, Section for Veterinary Diagnostics
Number of pages: 174
Publication date: 2006

Avian Influenza in wild birds: Evaluation of the risk of transmission to swine

General information
Publication status: Published
Organisations: Division of Microbiology and Risk Assessment, National Food Institute, Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Paisley, L., Vigre, H., Bøtner, A.
Publication date: 2006

Publication information
Publisher: Danmarks Fødevareforskning
Original language: English
Source: orbit
Source-ID: 242218
Research output: Book/Report › Report – Annual report year: 2006 › Research

Correlation between the presence of neutralizing antibodies against porcine circovirus 2 (PCV2) and protection against replication of the virus and development of PCV2-associated disease

General information
Effect of PMWS pig serum and PCV2 specific serum on mortality and weight gain in PMWS affected herds

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Number of pages: 157
Publication date: 2006

Host publication information
Title of host publication: Proceeding of the 19th International Pig Veterinary Congress
Source: orbit
Source-ID: 241736
Research output: Chapter in Book/Report/Conference proceeding – Annual report year: 2006 – Research

Isolation and genetic characterization of new reassortant H1N2 swine influenza A virus from pigs in Denmark

General information
Publication status: Published
Organisations: Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Sektion for Eksotiske Virussygdomme, Division of Virology, Virology
Contributors: Hjulsager, C. K., Bragstad, K., Bøtner, A., Larsen, L. E.
Publication date: 2006
Peer-reviewed: Yes
Event: Poster session presented at 7th International Congress of Veterinary Virology, Lisboa, Portugal.
Source: orbit
Source-ID: 240806
Research output: Contribution to conference – Poster – Annual report year: 2006 – Research – peer-review

Isolation and genetic characterization of new reassortant H1N2 swine influenza A virus from pigs in Denmark

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Hjulsager, C. K., Bragstad, K., Bøtner, A., Larsen, L. E.
Publication date: 2006
Peer-reviewed: No
Event: Abstract from 7th International Congress of Veterinary Virology, Lisboa, Portugal.
Source: orbit
**New swine influenza A H1N2 reassortment found in Danish swine**

**General information**
Publication status: Published
Organisations: Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Sektion for Eksotiske Virussygdomme, Division of Virology, Division of Microbiology and Risk Assessment, National Food Institute, Section for Veterinary Epidemiology and public sector consultancy, Virology
Publication date: 2006
Peer-reviewed: No
Event: Abstract from 19th International Pig Veterinary Society Congress, Copenhagen, Denmark.
Source: orbit

**New swine influenza A H1N2 reassortment found in Danish swine**

**General information**
Publication status: Published
Organisations: Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Sektion for Eksotiske Virussygdomme, Division of Virology, Division of Microbiology and Risk Assessment, National Food Institute, Section for Veterinary Epidemiology and public sector consultancy, Virology
Publication date: 2006
Peer-reviewed: Yes
Event: Paper presented at 19th International Pig Veterinary Society Congress, Copenhagen, Denmark.
Source: orbit

**PMWS in Denmark: Epidemiology, diagnosis and control**

**General information**
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute, Division of Microbiology and Risk Assessment, Section for Veterinary Epidemiology and public sector consultancy, Division of Veterinary Diagnostics and Research
Publication date: 2006

**Host publication information**
Title of host publication: Proceeding of the 19th International Pig Veterinary Congress
Source: orbit
Source-ID: 241727
Research output: Chapter in Book/Report/Conference proceeding – Article in proceedings – Annual report year: 2006 – Research
PMWS in Denmark: Epidemiology, Diagnosis and Control: Merial White Book

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute, Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research
Pages: 9-22
Publication date: 2006

Host publication information
Title of host publication: Proceeding of the 19th International Pig Veterinary Congress
Source: orbit
Source-ID: 241741
Research output: Chapter in Book/Report/Conference proceeding – Annual report year: 2006 – Research

PMWS - Laboratory Diagnosis on Herd and Pig Level in a Danish Case-Control Study

General information
Publication status: Published
Organisations: Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Sektion for Eksotiske Virussygdomme, Division of Virology, Section for Veterinary Epidemiology and public sector consultancy
Number of pages: 270
Publication date: 2006

Host publication information
Title of host publication: Proceeding of the 19th International Pig Veterinary Congress
Source: orbit
Source-ID: 241728
Research output: Chapter in Book/Report/Conference proceeding – Article in proceedings – Annual report year: 2006 – Research

PMWS-laboratory diagnosis on herd and pig level in a Danish case-study

General information
Publication status: Published
Organisations: Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Division of Microbiology and Risk Assessment, National Food Institute, Sektion for Eksotiske Virussygdomme, Division of Virology, Section for Veterinary Epidemiology and public sector consultancy
Publication date: 2006
Peers-reviewed: Yes
Event: Abstract from 19th International Pig Veterinary Society Congress, Copenhagen, Denmark.
Source: orbit
Source-ID: 241477
Research output: Contribution to conference – Conference abstract for conference – Annual report year: 2006 – Research – peer-review

Risikovurdering gennemført af Danmarks Fødevareforskning vedr. effekten af at fjerne loftet på 500 dyreheduer pr. landbrugsejendom

General information
Publication status: Published
Organisations: Division of Microbiology and Risk Assessment, National Food Institute, Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute, Section of Poultry Diseases, Division of Poultry, Fish
and Fur Animals, Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research, Section for Veterinary Epidemiology and public sector consultancy
Publication date: 2006

Publication information
Publisher: Danmarks Fødevareforskning
Original language: Danish
Source: orbit
Source-ID: 240707
Research output: Book/Report › Report – Annual report year: 2006 › Research

Sammenhæng mellem besætningsforhold og PMWS sygdomsudbrud – foreløbige resultater

General information
Publication status: Published
Organisations: Section for Veterinary Epidemiology and public sector consultancy, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Division of Microbiology and Risk Assessment, National Food Institute, Section for Veterinary Diagnostics, Sektion for Eksotiske Virussygdomme, Division of Virology
Contributors: Okholm Nielsen, E., Enøe, C., Bækbo, P., Vigre, H., Jorsal, S. E. L., Bøtner, A.
Pages: 726
Publication date: 2006
Peer-reviewed: No

Publication information
Journal: Månedsbladet Svin
Original language: Danish
Source: orbit
Source-ID: 242217
Research output: Contribution to journal › Journal article – Annual report year: 2006 › Research

The effects of PMWS on productivity and clinical expression in Danish herds from a case-control study

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute, Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research
Number of pages: 159
Publication date: 2006

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Title of host publication: Proceeding of the 19th International Pig Veterinary Congress
Place of publication: Copenhagen
Source: orbit
Source-ID: 241724
Research output: Chapter in Book/Report/Conference proceeding › Article in proceedings – Annual report year: 2006 › Research

The effects of PMWS on productivity and clinical expression on Danish herds from a case-control study

General information
Publication status: Published
Organisations: Section for Veterinary Epidemiology and public sector consultancy, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Sektion for Eksotiske Virussygdomme, Division of Virology, Section for Veterinary Diagnostics
Publication date: 2006
Peer-reviewed: Yes
Event: Abstract from 19th International Pig Veterinary Society Congress, Copenhagen, Denmark.
Source: orbit
Source-ID: 241480
Research output: Contribution to conference › Conference abstract for conference – Annual report year: 2006 › Research › peer-review
Experimental reproduction of postweaning multisystemic wasting syndrome (PMWS) in pigs in Sweden and Denmark with a Swedish isolate of porcine circovirus type 2

An experimental model using 3-day-old snatch-farrowed colostrum-deprived piglets co-infected with porcine circovirus type 2 (PCV2) and porcine parvovirus (PPV) is at present one of the best methods to study factors affecting development of postweaning multisystemic wasting syndrome (PMWS). A Swedish isolate of PCV2 (S-PCV2) retrieved in 1993 from a healthy pig has been used in this model to reproduce PMWS in pigs from Northern Ireland. This virus has been present in the Swedish pig population for at least a decade without causing any known PMWS disease problems, despite its potential pathogenicity. The reasons for this are unknown, but could be related to genetics, absence of triggers for PCV2 upregulation (infectious agent and/or management forms) within Swedish pig husbandry. In order to confirm the pathogenicity of S-PCV2, Swedish and Danish pigs were experimentally infected with this isolate according to the established model. Swedish pigs were also infected with a reference isolate of PCV2 (PCV2-1010) to compare the severity of disease caused by the two isolates in Swedish pigs. Both Danish and Swedish pigs developed PMWS after the experimental infection with S-PCV2. Antibodies to PCV2 developed later and reached lower levels in serum from pigs infected with S-PCV2 than in pigs inoculated with PCV2-1010. In general, pigs infected with S-PCV2 showed more severe clinical signs of disease than pigs infected with PCV2-1010, but pigs from all PCV2-inoculated groups displayed gross and histological lesions consistent with PMWS. All pigs inoculated with PPV, alone or in combination with PCV2, displayed interleukin-10 responses in serum while only pigs infected with PPV in combination with PCV2 showed interferon-a in serum on repeated occasions. Thus, the pathogenicity of S-PCV2 was confirmed and a role for cytokines in the etiology of PMWS was indicated.
Sammenhæng mellem besætningsforhold og PMWS sygdomsudbrud – foreløbige resultater

General information
Publication status: Published
Organisations: Section for Veterinary Epidemiology and public sector consultancy, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Division of Microbiology and Risk Assessment, National Food Institute, Section for Veterinary Diagnostics, Sektion for Eksotiske Virussygdomme, Division of Virology
Contributors: Okholm Nielsen, E., Enøe, C., Bækbo, P., Vigre, H., Jorsal, S. E. L., Bøtner, A.
Pages: 726
Publication date: 2005
Peer-reviewed: No

Publication information
Journal: Landudvalget for Svin
Original language: English
Source: orbit
Source-ID: 242215
Research output: Contribution to journal › Journal article – Annual report year: 2005 › Research

Spatial and temporal patterns of pig herds diagnosed with Postweaning Multisystemic Wasting Syndrome (PMWS) during the first two years of its occurrence in Denmark
The clinical syndrome Postweaning Multisystemic Wasting Syndrome (PMWS) in pigs has emerged globally during the last decade. In October 2001, the first pig herd diagnosed with PMWS was reported in Denmark, and since then the number of herds diagnosed with PMWS has increased markedly. The etiology of PMWS is not well understood, but increased knowledge of the causal factors is prerequisite for applying preventive interventions. In this study we described the temporal (time of diagnosis), spatial (location of herds) and spatio-temporal pattern of Danish pig herds diagnosed with PMWS during the first two years after the first herd was diagnosed, and we tested for spatial and spatio-temporal clustering using scan statistics. The study population consisted of pig herds that during the study period (October 2001 - September 2003) performed diagnostic submissions to the two major veterinary diagnostic laboratories in Denmark (6724 herds). Of these, 277 herds were diagnosed with PMWS. Two statistically significant spatial clusters of herds diagnosed with PMWS were identified. These clusters included 11% and 8% of the study herds, respectively. Within these two clusters the relative risk for a herd to be diagnosed with PMWS was twice as high as expected. One statistically significant spatio-temporal cluster was identified between February and May 2002. We discuss different hypotheses that could explain why pig herds diagnosed with PMWS were clustered both spatially and spatio-temporally, and conclude that the results support the hypothesis that PMWS is caused by introduction of a new, unidentified, pathogen into the Danish pig production. (c) 2005 Elsevier B.V. All rights reserved.

General information
Publication status: Published
Organisations: Division of Microbiology and Risk Assessment, National Veterinary Institute, Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research, Sektion for Eksotiske Virussygdomme, Division of Virology
Pages: 17-26
Publication date: 2005
Peer-reviewed: Yes

Publication information
Journal: Veterinary Microbiology
Volume: 110
Issue number: 1-2
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Scopus rating (2005): SJR 1.089 SNIP 1.258
Web of Science (2005): Indexed yes
Original language: English
DOIs:
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Source: orbit
Source-ID: 239904
A real-time RT-PCR SYBR Green-I assay for detection of porcine reproductive and respiratory syndrome virus

General information
Publication status: Published
Organisations: Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Section of Poultry Diseases, Division of Poultry, Fish and Fur Animals, Virology, Stald/vægterservice, Division of Virology, Sektion for Eksotiske Virussygdomme
Contributors: Hjulsager, C. K., Jørgensen, P. H., Larsen, L. E., Storgaard, T., Bøtner, A.
Publication date: 2004
Peer-reviewed: Yes
Event: Poster session presented at International qPCR Symposium & Application Workshop, Freising-Weihenstephan, Germany.
Source: orbit
Source-ID: 240993

Behandling med serum i PMWS besætninger

General information
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Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute, Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research, Division of Microbiology and Risk Assessment, National Food Institute
Publication date: 2004
Peer-reviewed: No
Publication information
Journal: DVHS efterårsmøde
Original language: Danish
Source: orbit
Source-ID: 242211

Behandling med serum i PMWS besætninger

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute, Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research, Division of Microbiology and Risk Assessment, National Food Institute
Pages: 675
Publication date: 2004
Peer-reviewed: No
Publication information
Journal: LU meddelelse
Original language: Danish
Source: orbit
Source-ID: 242210

DFVF indfører en ny serologiske metode til undersøgelse for antistoffer mod PCV2 fra 1.12.04

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Klausen, J., Nielsen, J., Bøtner, A.
Publication date: 2004
Peer-reviewed: No
DNA vaccination of pigs with open reading frame 1-7 of PRRS virus

We cloned all open reading frames of a Danish isolate of porcine reproductive and respiratory syndrome (PRRS) virus in DNA vaccination vectors. Pigs were vaccinated using a gene gun with each single construct (ORF1, ORF2, ORF3, ORF4, ORF5, ORF6, or ORF7) or combinations thereof. Vaccination with ORF7 consistently induced antibodies after three vaccinations, while antibodies were only sporadically detected in the remaining groups. After six vaccinations, all pigs were inoculated with PRRS virus and the post-inoculation antibody response was studied. Pigs vaccinated with ORF1 or ORF4 were primed for antibody response against NSP2 or GP4, respectively. Neutralising antibodies were detected in all pigs, with ORF5 vaccinated pigs showing the highest titres.

Experimental airborne transmission of PRRS virus

A series of three experiments, differing primarily in airflow volume, were performed to evaluate the likelihood of airborne transmission of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) from infected to non-infected pigs. Pigs were housed in two units (unit A and unit B) located 1 m apart and connected by pipes. The air pressure and diameter of the pipes, depending on experiments, were strictly controlled to allow desired airflow volumes from unit A to unit B. Either 25 (experiment 1 and experiment 3) or 26 (experiment 2) pigs infected recently with PRRSV, and either 25 (experiment 1 and experiment 3) or 17 (experiment 2) pigs from a PRRSV-free herd, were housed in unit A. Either 50 pigs (experiment 1 and experiment 3) or 43 pigs (experiment 2) from a PRRSV-free herd were housed in unit B. The amount of air transmitted from unit A to unit B, expressed as a percentage of ventilation intake, was approximately 70, 10, and 1% for experiment 1, experiment 2 and experiment 3, respectively. Blood samples were collected from all pigs once per week and analyzed for antibodies against PRRSV. Based on these methods, airborne transmission of PRRSV from infected to non-infected pigs was confirmed in each of the three experiments.
Immunisation against PCV2 structural protein by DNA vaccination of mice

Porcine circovirus type 2 (PCV2) is the causative agent of an emerging swine disease, postweaning multisystemic wasting syndrome (PMWS). The disease affects primarily 5-12-weeks-old pigs which might suggest that infection with PCV2 occurs when the level of maternal antibodies have declined to sub-protective levels around weaning at 3-5-weeks of age. If immunoprophylaxis is to be effective, an immunisation method capable of breaking through maternal immunity must be employed. In this study, we have developed and investigated the potential of a DNA vaccination approach to be one such method. The gene encoding the capsid protein of PCV2 was cloned in a DNA vaccination plasmid and expression of capsid protein was demonstrated in vitro. Mice were gene gun vaccinated three times and all mice responded serologically by raising antibodies against PCV2. The results suggest, that DNA based vaccination might offer opportunities for vaccination of piglets against PCV2.

Influence of routes and administration parameters on antibody response of pigs following DNA vaccination

Using the nucleoprotein of porcine reproductive and respiratory syndrome virus as model antigen, we optimised parameters for gene gun vaccination of pigs, including firing pressure and vaccination site. As criteria for optimisation, we characterised particle penetration and local tissue damage by histology. For selected combinations, vaccination efficiency in terms of antibody response was studied. Gene gun vaccination on ear alone was as efficient as a multi-site (ear, thorax, inguinal area, tongue mucosa) gene gun approach, and more efficient than combined intramuscular (i.m./intradermal (i.d.) injection of plasmid DNA. This indicates, that the ear is an attractive site for gene gun vaccination of pigs.
Interlaboratory testing of porcine sera for antibodies to porcine circovirus type 2

A panel of 20 porcine sera was distributed to 5 laboratories across Europe and Canada. Each center was requested to test the sera for the presence of porcine circovirus type 2 antibodies using the routine assays, indirect immunofluorescence assay (IFA) and indirect immunoperoxidase monolayer assay (IPMA), and to determine the titer of each serum. Results from all centers were then compiled and correlated. They demonstrate a wide variation in the titers obtained between laboratories. These differences were dependent on the assay used and the choice of fixative. In general, IPMA gave higher titers than did IFA, and paraformaldehyde gave higher titers than did acetone or ethyl alcohol. This report highlights the need for standardized procedures and biologicals for this virus.

General information
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Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: McNair, I., Marshall, M., McNeilly, F., Bøtner, A., Ladekjær-Mikkelsen, A., Vincent, I., Herrmann, B., Sanchez, R., Rhodes, C.
Pages: 164-166
Publication date: 2004
Peer-reviewed: Yes

PMWS: Experimental model and co-infections
Porcine circovirus type 2 (PCV2) is now recognised as the causal agent of porcine multisystemic wasting syndrome (PMWS), an economically important wasting disease of young pigs [J. Vet. Diagn. Invest. 12 (2000) 3]. Gross lesions of PMWS include generalised lymphadenopathy, hepatitis, nephritis and pneumonia and typical histological lesions include lymphocytic depletion and multinucleated giant cell formation in lymph nodes, degeneration and necrosis of hepatocytes, and multifocal lymphohistocytic interstitial pneumonia. This communication will review the results of experimental infections of gnotobiotic (GN), colostrum-deprived (CD) and colostrum-fed (CF) pigs within our group, and elsewhere, with PCV2 and the conclusions that can be drawn from this work.

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Allan, G. M., McNeilly, F., Ellis, J., Krakowka, S., Bøtner, A., McCullough, K., Nauwynck, H., Kennedy, S., Meehan, B., Charreyre, C.
Pages: 165-168
Publication date: 2004
Peer-reviewed: Yes

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Journal: Veterinary Microbiology
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ISSN (Print): 0378-1135
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Scopus rating (2004): SJR 0.873 SNIP 1.247
Web of Science (2004): Indexed yes
Original language: English
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10.1016/j.vetmic.2003.10.009
Source: orbit
Source-ID: 241322
Research output: Contribution to journal › Journal article – Annual report year: 2004 › Research › peer-review

Rapport om laboratorieberedskabsøvelse vedrørende mund- og klovesyge

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A.
Publication date: 2004

Publication information
Publisher: Fødevarestyrelsen
Original language: Danish
Source: orbit
Source-ID: 242212
Research output: Book/Report › Report – Annual report year: 2004 › Research

Transmission of PMWS

General information
Publication status: Published
Organisations: Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Sektion for Eksotiske Virussygdomme, Division of Virology
Publication date: 2004
Peer-reviewed: Yes
Event: Abstract from 18th International Pig Veterinary Society Congress, Hamburg, Germany.
Source: orbit
Source-ID: 241474
Research output: Contribution to conference › Conference abstract for conference – Annual report year: 2004 › Research › peer-review

Airborne transmission of Porcine Reproductive and Respiratory Syndrome Virus between pig units located at close range

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute, Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research
Contributors: Kristensen, C. S., Bætner, A., Nielsen, J. P., Jorsal, S. E. L.
Publication date: 2003
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Source: orbit
Source-ID: 241706
Årsagsforhold ved svinesygdommen PMWS

General information
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Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A., Bækbo, P.
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Original language: Danish
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Association of lymphopenia with porcine circovirus type 2 induced postweaning multisystemic wasting syndrome (PMWS)

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Nielsen, J., Vincent, I., Ladekjær-Mikkelsen, A., Allan, G., McCullough, K., Bøtner, A.
Pages: 97-111
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Journal: Veterinary Immunology and Immunopathology
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Original language: English
Source: orbit
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Characterisation of The First Cases of PMWS in Denmark

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute, Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research
Publication date: 2003
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Diagnostik og forekomst af PMWS
DNA vaccination using porcine circovirus type 2 structural protein

General information
Publication status: Published
Organisations: National Veterinary Institute, Sektion for Eksotiske Virussygdomme, Division of Virology
Publication date: 2003
Peer-reviewed: No
Event: Abstract from 6th International Congress of Veterinary Virology, St. Malo, France.
Source: orbit
Source-ID: 241342
Research output: Contribution to conference » Conference abstract for conference – Annual report year: 2003 » Research

Generation of an infectious clone of VR-2332, a highly virulent North American type isolate of porcine reproductive and respiratory syndrome virus

A full-length cDNA clone of the prototypical North American porcine reproductive and respiratory syndrome virus (PRRSV) isolate VR-2332 was assembled in the plasmid vector pOK(12). To rescue infectious virus, capped RNA was transcribed in vitro from the pOK(12) clone and transfected into BHK-21C cells. The supernatant from transfected monolayers were serially passaged on Marc-145 cells and porcine pulmonary alveolar macrophages. Infectious PRRSV was recovered on Marc-145 cells as well as porcine pulmonary macrophages; thus, the cloned virus exhibited the same cell tropism as the parental VR-2332 strain. However, the cloned virus was clearly distinguishable from the parental VR-2332 strain by an engineered marker, a BstZ171 restriction site. The full-length cDNA clone had 11 nucleotide changes, 2 of which affected coding, compared to the parental VR-2332 strain. Additionally, the transcribed RNA had an extra G at the 5' end. To examine whether these changes influenced viral replication, we examined the growth kinetics of the cloned virus in vitro. In Marc-145 cells, the growth kinetics of the cloned virus reflected those of the parental isolate, even though the titers of the cloned virus were consistently slightly lower. In experimentally infected 5.5-week-old pigs, the cloned virus produced blue discoloration of the ears, a classical clinical symptom of PRRSV. Also, the seroconversion kinetics of pigs infected with the cloned virus and VR-2332 were very similar. Hence, virus derived from the full-length cDNA clone appeared to recapitulate the biological properties of the highly virulent parental VR-2332 strain. This is the first report of an infectious cDNA clone based on American-type PRRSV. The availability of this cDNA clone will allow examination of the molecular mechanisms behind PRRSV virulence and attenuation, which might in turn allow the production of second-generation, genetically engineered PRRSV vaccines.

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Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Nielsen, H., Liu, G., Nielsen, J., Oleksiewicz, M., Bøtner, A., Storgaard, T., Faaberg, K.
Pages: 3702-3711
Publication date: 2003
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Publication information
Journal: Journal of Virology
Volume: 77
Issue number: 6
In utero infection with porcine reproductive and respiratory syndrome virus modulates leukocyte subpopulations in peripheral blood and bronchoalveolar fluid of surviving piglets

It is well known that piglets congenitally infected with porcine reproductive and respiratory syndrome virus (PRRSV) can be viremic at birth, and that preweaning mortality due to secondary infections often increases during acute outbreaks of PRRS. Therefore, an immunosuppressive effect of in utero infection has been suggested. The aim of the present study was to characterise the changes of leukocyte populations in piglets surviving in utero infection with PRRSV. A total of 27 liveborn uninfected control piglets and 22 piglets infected transplacentally with a Danish strain of PRRSV were included. At 2 and 4 weeks of age, 21 of 22 (96%) and 7 of 14 (50%) examined infected piglets were still viremic, whereas PRRSV could not be detected in the six infected piglets examined at 6 weeks of age. Flow cytometry analysis was used to determine the phenotypic composition of leukocytes in peripheral blood and bronchoalveolar lavage fluid (BALF) of 2-, 4- and 6-week-old infected piglets and age-matched uninfected controls. The key observation in the present study is that high levels of CD8+ cells constitute a dominant feature in peripheral blood and BALF of piglets surviving in utero infection with PRRSV. In BALF, the average high level of CD8+ cells in 2-week-old infected piglets (33.4±12.6%) was followed by a decline to 7.3±3.0% and 11.1±3.0% at 4 and 6 weeks of age. BALF of control piglets contained 1.6±0.9, 2.3±1.8 and 1.9±0.5% CD8+ cells, only. In peripheral blood, however, the average number of CD8+ cells remained at high levels in the infected piglets throughout the post-natal experimental period (2.8±1.9, 2.9±1.8 and 3.2±1.7×106 CD8+ cells/ml at 2, 4 and 6 weeks, respectively). In the controls, the average levels of CD8+ cells were 0.9±0.2, 1.9±1.7 and 1.6±0.5×106/ml, respectively. Furthermore, the numbers of CD2+, CD4+CD8+ and SLA-classII+ cells, respectively, in peripheral blood, together with the levels of CD2+ and CD3+ cells in BALF were increased in the infected piglets infected in utero compared to the uninfected controls. The kinetic analyses carried out in the present study reflect that in utero infection with PRRSV modulates immune cell populations in peripheral blood and BALF of surviving piglets. The observed changes are characterised by high levels of CD8+ cells supporting an important role of these cells in PRRSV infection. The present results, however, do not support the existence of post-natal immunosuppression following in utero infection with PRRSV.

In utero infection with PRRSV affects immune functions of surviving piglets

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Nielsen, J., Bøtner, A., Tingstedt, J. E., Aasted, B., Johnsen, C. K., Riber, U., Lind, P.
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Source: orbit
Source-ID: 241333
Research output: Contribution to journal › Journal article – Annual report year: 2003 › Research › peer-review
Serological profiles in Danish PMWS case and control herds

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Ladekjær-Mikkelsen, A., Bøtner, A., Nielsen, J., Hassing, A., Bækbo, P.
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Event: Abstract from 4th International Symposium on Emerging and Re-emerging Pig Diseases, Rome, Italy.
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Serologiske profiler for PCV2 og PPV i danske besætninger med og uden Postweaning Multisystemic Wasting Syndrome (PMWS)

General information
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Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Hassing, A., Bækbo, P., Bøtner, A., Ladekjær-Mikkelsen, A.
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Peer-reviewed: No

Serum Treatment to Prevent PMWS in Pigs Experimentally Infected with PCV2 and PRRSV

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Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute, Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research
Contributors: Ladekjær-Mikkelsen, A. S., Nielsen, J., Bille-Hansen, V., Bøtner, A.
Publication date: 2003
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Event: Abstract from 6th International Congress of Veterinary Virology, St. Malo, France.
Source-ID: 241718

Sundhed og produktion hos svin i multisitesystemer

General information
Publication status: Published
Organisations: Division of Microbiology and Risk Assessment, National Food Institute, Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute, Secretariat, Division of Veterinary Diagnostics and Research
Airborne transmission of A. pleuropneumoniae and PRRS virus between pig units

General information
 Publication status: Published
 Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute, Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research, Secretariat, Division of Microbiology and Risk Assessment, National Food Institute
 Contributors: Kristensen, C. S., Bøtner, A., Angen, Ø., Sørensen, V., Jorsal, S. E. L., Takai, H., Barfod, K., Nielsen, J. P.
 Pages: 272-272
 Publication date: 2002

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 Research output: Chapter in Book/Report/Conference proceeding – Annual report year: 2002 › Research › peer-review

Comparison of pathogenic effects of different PCV2 inocula on development of PMWS

General information
 Publication status: Published
 Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
 Contributors: Ladekjær-Mikkelsen, A., Nielsen, J., Krakowka, S., Ellis, J., McNeilly, F., Allan, G., Bøtner, A.
 Publication date: 2002
 Peer-reviewed: No
 Event: Abstract from 17th International Pig Veterinary Society Congress, Ames, Iowa, United States.
 Source: orbit
 Source-ID: 241766
 Research output: Contribution to conference › Conference abstract for conference – Annual report year: 2002 › Research

Cytokine mRNA profiles in bronchoalveolar cells of piglets experimentally infected in utero with porcine reproductive and respiratory syndrome virus: Association of sustained expression of IFN-gamma and IL-10 after viral clearance

An experimental model was used to investigate mRNA cytokine profiles in bronchoalveolar cells (BALC) from piglets, infected in utero with porcine reproductive and respiratory syndrome virus (PRRSV). The BALC's were analyzed for the cytokines TNF-alpha, IFN-gamma, IL-8, IL-10, and IL-12(p40) by real-time TaqMan polymerase chain reaction in 2-, 4-, and 6-week-old piglets, respectively. High levels of IFN-gamma mRNA was detected in all piglets, while IL-10 was upregulated in 2-week-old piglets, was at normal levels in 4-week-old piglets, and elevated again in 6-week-old piglets. IL-12 was weakly elevated in all three age groups. Virus was reduced by 50% in 4-week-old piglets and cleared by 6 weeks of age. The sustained expression of IFNgamma and reduction of IL-10 production indicate an important role for these cytokines in immunity to PRRSV.
Cytokine mRNA profiles in bronchoalveolar cells of piglets experimentally infected in utero with porcine reproductive and respiratory syndrome virus: Association of sustained expression of IFN-gamma and IL-10 after viral clearance

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute, Section for Veterinary Epidemiology and public sector consultancy, Division of Veterinary Diagnostics and Research
Contributors: Johnsen, C., Bøtner, A., Kamstrup, S., Lind, P., Nielsen, J.
Pages: 549-556
Publication date: 2002
Peer-reviewed: Yes

Cytokine mRNA profiles in the lungs of piglets experimentally infected in utero with porcine reproductive and respiratory syndrome virus (PRRSV): Evidence for sustained expression of IFN-G and IL-10 after viral clearance from the lungs

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute, Section for Veterinary Epidemiology and public sector consultancy, Division of Veterinary Diagnostics and Research
Contributors: Johnsen, C. K., Bøtner, A., Kamstrup, S., Lind, P., Nielsen, J.
Publication date: 2002
Peer-reviewed: No
Source: orbit
Source-ID: 241689
Research output: Contribution to conference > Conference abstract for conference – Annual report year: 2002 > Research

Dyr som influenzareservoir
Experimental inoculation of late term pregnant sows with a field isolate of porcine reproductive and respiratory syndrome vaccine-derived virus

The use of a live attenuated porcine reproductive and respiratory syndrome virus (PRRSV) vaccine in piglets has been associated with reproductive disorders in non-vaccinated sows. Vaccine-derived virus (VDV) has been isolated from foetuses, stillborn pigs, and dead piglets, indicating that the live vaccine spread from vaccinated piglets to non-vaccinated sows, and that the virus might be implicated in the severe reproductive problems observed. In the present study, one such VDV isolate was used to experimentally infect pregnant sows in the last trimester. The chosen isolate, which had more than 99.6% identity to the attenuated vaccine virus, originated from the lungs of a stillborn pig from a swine herd with a sudden high level of stillborn pigs and increased piglet mortality in the nursing period. Intranasal inoculation of sows with the virus isolate resulted in congenital infection, foetal death, and preweaning pig mortality. As such, the present study showed that vaccine-derived PRRSV can cause disease in swine consistent with PRRS.

Influenza A virus i et zoonotisk perspektiv

Influenza A virus is a zoonotic virus, which means that it can be transmitted from animals to humans. Understanding the zoonotic potential of influenza A virus is crucial for public health, as it can lead to outbreaks in human populations. This article discusses the zoonotic aspects of influenza A virus, including its ability to cross species barriers and cause disease in both animal and human hosts. The authors review the current knowledge on influenza A virus transmission and highlight the importance of surveillance and preventive measures to mitigate the risk of zoonotic influenza outbreaks.
PMWS

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A.
Publication date: 2002
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Original language: English
Source: orbit
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Research output: Contribution to journal › Journal article – Annual report year: 2002 › Communication

PMWS Hvordan stilles diagnosen Post Weaning Multistemic Wasting Syndrome ?

General information
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Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute, Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research
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Original language: Danish
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Research output: Contribution to journal › Journal article – Annual report year: 2002 › Research

PMWS. Hvordan stilles diagnosen Postweaning Multisystemic Wasting Syndrome

General information
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Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute, Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research
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Publication date: 2002
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Journal: Dansk Veterinaertidsskrift
Volume: 85
Issue number: 4
ISSN (Print): 0106-6854
Original language: English
Source: orbit
Source-ID: 241102
Porcine B-cells recognize epitopes that are conserved between the structural proteins of American- and European-type porcine reproductive and respiratory syndrome virus

By selecting phage display libraries with immune sera from experimentally infected pigs, porcine B-cell epitopes in the open reading frame (ORF) 2, 3, 5 and 6 proteins of European-type porcine reproductive and respiratory syndrome virus (PRRSV) were identified. The sequences of all the epitopes were well conserved in European-type PRRSV and even between European- and American-type PRRSV. Accordingly, sera from pigs infected with American-type PRRSV cross-reacted with the European-type epitopes. Thus, this study showed, for the first time, the presence of highly conserved epitopes in the matrix protein and envelope glycoproteins of PRRSV. ORF5 and 6 epitopes localized to protein parts that are predicted to be hidden in PRRSV virions. In contrast, ORF2 and 3 epitopes localized to putative protein ectodomains. Due to the interesting localization, the sequence surrounding the ORF2 and 3 epitopes was subjected to closer scrutiny. A heptad motif, VSRRRIYQ, which is present in a single copy in ORF2 and 3 proteins, was identified; this arrangement is completely conserved in all European-type PRRSV sequences available. The VSRRRIYQ repeat motif colocalized closely with one of the ORF2 epitopes and secondary structure modelling showed that this segment of the ORF2 protein could form an amphipathic helix. Intriguingly, a mutation associated with virulence/attenuation of an American vaccine strain of PRRSV also localized to this ORF2 protein segment and affected the hydrophobic face of the predicted amphipathic helix. Further work is needed to determine whether these findings delineate a functional domain in the PRRSV ORF2 protein.
Reproduction of postweaning multisystemic wasting syndrome (PMWS) in immunostimulated and non-immunostimulated 3-week-old piglets experimentally infected with porcine circovirus type 2 (PCV2)

Postweaning multisystemic wasting syndrome (PMWS) in swine is causally associated with the newly recognised pathogen, porcine circovirus type 2 (PCV2). In this study, 3-week-old SPF PCV2-seronegative piglets were inoculated intranasally with PCV2. The effect of immunostimulation on the induction of PMWS was investigated by immunisation with keyhole limpet hemocyanin (KLH) emulsified in incomplete Freund’s adjuvant. The study was terminated 5 weeks after inoculation. While disease was not observed in the age-matched controls, two out of five non-immunised PCV2-infected piglets died on postinoculation day (PID) 21, and one was euthanized on PID 25 in moribund condition. These animals had appeared lethargic with persistent fever from PID 12 onwards. The euthanized pig appeared smaller than littermates and suffered from jaundice. At postmortem examination, gastric ulceration, icterus, and liver and thymus atrophy were observed. Furthermore, histological lesions of degenerating hepatocytes and hepatitis in combination with lymphoid depletion and mononuclear cells in lymph nodes were consistent with the diagnosis of PMWS. One out of five immunostimulated PCV2-infected piglets was euthanized on PID 22 with convulsions after a period with wasting. This pig was lethargic from PID 14 onwards with persistent fever from PID 8 and transient dyspnoea. No differences in clinical signs, gross pathologic or histological findings were observed for the remaining non-immunostimulated and immunostimulated PCV2-infected piglets. All 10 PCV2-inoculated piglets seroconverted to PCV2 within 14 days after inoculation. By virus isolation, quantitative polymerase chain reaction (Q-PCR), and immunostaining of cryostat sections, it was demonstrated that lymphoid tissue contained abundant PCV2 antigen. Viral DNA load in serum samples was assessed by Q-PCR. All four PMWS-affected piglets had high levels of PCV2 DNA in serum, suggesting that there was a correlation between high levels of viral DNA in serum and the development of PMWS. In conclusion, infection with PCV2 caused PMWS in SPF piglets, however, the immunostimulation did not seem to play a critical role.

General information
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Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute, Stald/vægterservice, Technical University of Denmark
Contributors: Ladekjær-Mikkelsen, A., Nielsen, J., Stadejek, T., Storgaard, T., Krakowka, S., Ellis, J., McNeilly, F., Allan, G., Bøtner, A.
Pages: 97-114
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Peer-reviewed: Yes

Publication information
Journal: Veterinary Microbiology
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Status på PMWS situationen i Danmark

General information
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Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute, Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research
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Journal: Hyologisk
Volume: 07
ISSN (Print): 0906-0995
Original language: Danish
The genetic diversity of European type PRRSV is similar to that of the North American type but is geographically skewed within Europe

Porcine reproductive and respiratory syndrome virus (PRRSV) is a recently emerged pathogen. Two PRRSV genotypes exist, North American and European, which are only 55-70% identical at the nucleotide level. Previous studies have shown high nucleotide diversity in the North American genotype and low nucleotide diversity in the European genotype. Here, we analyzed the ORF5 and ORF7 genes for a large number of new European type PRRSV isolates in conjunction with existing database sequences. This new analysis showed that contrary to previous assumptions, genetic diversity is at least as high in the European genotype as in the North American genotype. Furthermore, we showed that genetic diversity of European type PRRSV has a marked geographical pattern, with exceptionally high genetic diversity among Italian sequences. The geographical pattern of diversity in relation to the epidemiology of PRRSV in Europe is discussed. Discrepancies between ORF5- and ORF7-based genealogies were observed, and further analysis of the data set confirmed the presence of recombination. We were therefore able to report the first observation of recombination in wild-type isolates of European genotype PRRSV

A field case of transplacental infection with PCV-2 associated with reproductive failure

A field case of transplacental infection with PCV-2 associated with reproductive failure
A molecular clock dates the common ancestor of European-type porcine reproductive and respiratory syndrome virus at more than 10 years before the emergence of disease

The disease caused by porcine reproductive and respiratory syndrome virus (PRRSV) emerged independently and almost simultaneously in Europe (1990) and North America (1987). The original reservoir of the virus and the date it entered the pig populations is not known. In this study, we demonstrate an accurate molecular clock for the European PRRSV ORF 3 gene, place the root in the genealogy, estimate the rate of nucleotide substitution, and date the most recent common viral ancestor of the data set to 1979; more than 10 years before the onset of the European epidemic. Based on these findings, we conclude that PRRSV virus most likely entered the pig population some time before the epidemic emergence of the virus, and hence, that emergence of European-type PRRSV is not the result of a recent species transmission event. Together, our results show that ORF3 sequencing is a valuable epidemiologic tool for examining the emergence and spread of PRRSV in Europe. As such, the panel of well-characterized and highly divergent ORF3 sequences described in this study provides a reference point for future molecular epidemiologic studies.

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Organisations: Sektion for Eksotiske Virusygdomme, Division of Virology, National Veterinary Institute
Contributors: Forsberg, R., Oleksiewicz, M. B., Krabbe Petersen, A. M., Hein, J., Bøtner, A., Storgaard, T.
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Journal: Virology
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Scopus rating (2001): SJR 1.751 SNIP 0.907
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Source: orbit
Source-ID: 230639
Research output: Contribution to journal › Journal article – Annual report year: 2001 › Research › peer-review

Epitope mapping porcine reproductive and respiratory syndrome virus by phage display: the nsp2 fragment of the replicase polyprotein contains a cluster of B-cell epitopes

We screened phage display libraries of porcine reproductive and respiratory syndrome virus (PRRSV) protein fragments with sera from experimentally infected pigs to identify linear B-cell epitopes that are commonly recognized during infection in vivo. We identified 10 linear epitope sites (ES) 11 to 53 amino acids in length. In the replicase polyprotein, a total of eight ES were identified, six of which localized to the Nsp2 replicase polyprotein processing end product, In the structural proteins, a total of two ES were identified, in the ORF3 and ORF4 minor envelope glycoproteins, The ORF4 ES was previously identified by monoclonal antibody mapping (J. J, M. Meulenberg, A. P. van Nieuwstadt, A, van Essen-Zandenbergen, and J, P, M, Langeveld, J. Virol, 71:6061-6067, 1997), but its immunogenicity had not been examined in pigs. We found that six experimentally PRRSV-infected pigs consistently had very high antibody titers against the ORF4 ES, In some animals, sera diluted 1:62,500 still gave weak positive enzyme immunoassay reactivity against the ORF4 ES, This hitherto unrecognized immunodominance likely caused phages displaying the ORF4 ES to outcompete phages displaying other ES during library screening with porcine sera and accounted for our failure to identify more than two ES in the structural genes of PRRSV, Genetic analysis showed that variable ES were also the most immunogenic in vivo.
Serological analysis indicated differences in the immunoglobulin A responses between short-term and longer-term viremic pigs towards some ES, The implications of these findings for PRRSV diagnostics and immunopathogenesis are discussed.

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Organisations: Sektion for Eksotiske Virusygdomme, Division of Virology, National Veterinary Institute,
Stald/vægterservice
Contributors: Oleksiewicz, M., Bøtner, A., Toft, P., Normann, P., Storgaard, T.
Pages: 3277-3290
Publication date: 2001
Epitope mapping Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) by phage display: The nsp2 fragment of the replicase polyprotein contains a cluster of B-cell epitopes

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
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Peer-reviewed: Yes

Experimental infection of 3-week-old piglets with PCV2 altered the level of various peripheral blood leukocyte populations

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Nielsen, J., Vincent, I., Ladekjær-Mikkelsen, A., Allan, G., McCullough, K., Bøtner, A.
Publication date: 2001
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Research output: Contribution to conference › Conference abstract for conference – Annual report year: 2001 › Research

High frequency RNA recombination in porcine reproductive and respiratory syndrome virus occurs preferentially between parental sequences with high similarity

Two types of porcine reproductive and respiratory syndrome virus (PRRSV) exist, a North American type and a European type. The co-existence of both types in some countries, such as Denmark, Slovakia and Canada, creates a risk of inter-type recombination. To evaluate this risk, cell cultures were co-infected with either a North American and a European type of PRRSV or two diverse types of European isolate. Subsequently, an approximately 600 bp region of the PRRSV genome was tested for recombination by quantitative real-time RT-PCR. Between 0-1 and 2-5% RNA recombination was found between the European isolates, but no recombination was detected between the European and North American types. Calculation of the maximum theoretical risk of European-American recombination, based on the sensitivity of the RT-PCR system, revealed that RNA recombination between the European and North American types of PRRSV is at least 10000 times less likely to occur than RNA recombination between diverse European isolates.
Monitoring porcine reproductive and respiratory syndrome virus infection status in swine herds based on analysis of antibodies in meat juice samples

An indirect ELISA test was developed as a novel tool aimed at monitoring the herd infection status of swine herds. Meat juice samples from pig carcasses were analysed for the presence of antibodies against porcine reproductive and respiratory syndrome virus (PRRSV). A study of samples from herds with known PRRS status was undertaken. The PRRS status of the herds was evaluated based on the analysis of blood samples by another serological test (blocking ELISA) capable of differentiating between infection with PRRSV of the American type and European type. The specificity of the indirect ELISA test on meat juice samples was 0.98. The sensitivity of the test depended on the type of the PRRSV strain involved. The apparent prevalence in herds infected with the American type of PRRSV was 0.44. The apparent prevalence in herds infected with the European type of PRRSV was 0.64. Herd level sampling and herd level criteria for assessing the PRRS status of herds by the new test were developed. Herds were classified as PRRS negative or PRRS seropositive based on 10 meat juice samples collected randomly at slaughter throughout a 3-month-period. Herd PRRS status classification by the indirect ELISA was validated in 47 herds by collection of blood samples from the herds. Eighteen herds were classified as PRRS negative by both test systems. Twenty-nine herds were classified as PRRS seropositive by both test systems. Acceptable herd classification was achieved using this test.
Ny sygdom hos grise i Danmark

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Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute, Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research
Contributors: Bøtner, A., Jorsal, S. E. L.
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Journal: Fødevareministeriets eksterne ugebrev
Issue number: 25
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Source: orbit
Source-ID: 242198
Research output: Contribution to journal › Journal article – Annual report year: 2001 › Research

PCV2 as the causal agent of PMWS

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Allan, G., Pensaert, M., Bøtner, A., McCullough, K., Krakowka, S., Ellis, J.
Publication date: 2001
Peer-reviewed: No

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Research output: Contribution to journal › Letter – Annual report year: 2001 › Research

PMWS in Denmark?

General information
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Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute, Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research
Publication date: 2001
Peer-reviewed: No
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Research output: Contribution to conference › Conference abstract for conference – Annual report year: 2001 › Research

PMWS in Denmark?

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Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute, Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research
Publication date: 2001
Peer-reviewed: Yes
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Source-ID: 241469
Research output: Contribution to conference › Conference abstract for conference – Annual report year: 2001 › Research › peer-review
Porcin dermatitis og nephropati syndrom - ny svinesygdom i Danmark

General information
Publication status: Published
Organisations: Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Sektion for Eksotiske Virussygdomme, Division of Virology
Contributors: Jensen, T. K., Jorsal, S. E. L., Bøtner, A.
Pages: 8-9
Publication date: 2001
Peer-reviewed: No

Publication information
Journal: SVS/SVIV Information
Volume: 72
Original language: English
Source: orbit
Source-ID: 241427
Research output: Contribution to journal › Journal article – Annual report year: 2001 › Research

Porcin circovirus type 2 (PCV2) infektion i svin

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Ladekjær-Mikkelsen, A., Bøtner, A.
Publication date: 2001

Publication information
Publisher: SVS/SVIV
Original language: English
Source: orbit
Source-ID: 242203
Research output: Book/Report › Report – Annual report year: 2001 › Research

Post-weaning Multisystemic Wasting Syndrome (PMWS) i Danmark?

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute, Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research
Pages: 7-9
Publication date: 2001
Peer-reviewed: No

Publication information
Journal: SVS/SVIV Information
Original language: Danish
Source: orbit
Source-ID: 241432
Research output: Contribution to journal › Journal article – Annual report year: 2001 › Research

Post-weaning Multisystemic Wasting Syndrome: Quantification of porcine circovirus load by real-time PCR

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Ladekjær-Mikkelsen, A., Stadejek, T., Storgaard, T., Nielsen, J., Allan, G., Bøtner, A.
Publication date: 2001
Peer-reviewed: No
Rapport fra arbejdsgruppen vedrørende anvendelse af kødsaft til overvågning af husdyrsviruddomme og zoonoser i Danmark

General information
Publication status: Published
Organisations: Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Administration and Service, Division of Poultry, Fish and Fur Animals, Sektion for Eksotiske Virussygdomme, Division of Virology, Section of Poultry Diseases, Secretariat
Contributors: Angen, Ø., Bager, F., Bøtner, A., Jorsal, S. E. L., Jørgensen, P. H., Klausen, J., Sørensen, V., Uttenthal, Å.
Publication date: 2001

Reproduction of PMWS in immunostimulated and non-immunostimulated conventional 3-week-old piglets experimentally infected with PCV2

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A., Ladekjær-Mikkelsen, A., Nielsen, J., Krakowka, S., Ellis, J., McNeilly, F., Allan, G., Storgaard, T.
Publication date: 2001
Peer-reviewed: No
Source: orbit
Source-ID: 240421
Research output: Book/Report › Report – Annual report year: 2001 › Research

Reversion of a live porcine reproductive and respiratory syndrome virus vaccine investigated by parallel mutations
A live attenuated porcine reproductive and respiratory syndrome (PRRS) vaccine virus has been shown to revert to virulence under field conditions. In order to identify genetic virulence determinants, ORF1 from the attenuated vaccine virus and three Danish vaccine-derived field isolates was sequenced and compared with the parental strain of the vaccine virus (VR2332). This revealed five mutations that had occurred independently in all three vaccine-derived field isolates, indicating strong parallel selective pressure on these positions in the vaccine virus when used in swine herds. Two of these parallel mutations were direct reversions to the parental VR2332 sequence and were situated in a papain-like cysteine protease domain and in the helicase domain. The remaining parallel mutations might be seen as second-site compensatory mutations for one or more of the mutations that accumulated in the vaccine virus sequence during cell-culture adaptation. Evaluation of the remaining mutations in the ORF1 sequence revealed stronger selective pressure for amino acid conservation during spread in pigs than during vaccine production. Furthermore, it was found that the selective pressure did not change during the time period studied. The implications of these findings for PRRS vaccine attenuation and reversion are discussed.

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute, Stald/vægterservice, Technical University of Denmark
Contributors: Nielsen, H. S., Oleksiewicz, M., Forsberg, R., Stadejek, T., Bøtner, A., Storgaard, T.
Pages: 1263-1272
Publication date: 2001
Peer-reviewed: Yes

Publication information
Journal: Journal of General Virology
Volume: 82
Issue number: 6
ISSN (Print): 0022-1317
Reversion of a live porcine reproductive and respiratory virus vaccine investigated by parallel mutations

A live attenuated porcine reproductive and respiratory syndrome (PRRS) vaccine virus has been shown to revert to virulence under field conditions. In order to identify genetic virulence determinants, ORF1 from the attenuated vaccine virus and three Danish vaccine-derived field isolates was sequenced and compared with the parental strain of the vaccine virus (VR2332). This revealed five mutations that had occurred independently in all three vaccine-derived field isolates, indicating strong parallel selective pressure on these positions in the vaccine virus when used in swine herds. Two of these parallel mutations were direct reversions to the parental VR2332 sequence and were situated in a papain-like cysteine protease domain and in the helicase domain. The remaining parallel mutations might be seen as second-site compensatory mutations for one or more of the mutations that accumulated in the vaccine virus sequence during cell-culture adaptation. Evaluation of the remaining mutations in the ORF1 sequence revealed stronger selective pressure for amino acid conservation during spread in pigs than during vaccine production. Furthermore, it was found that the selective pressure did not change during the time period studied. The implications of these findings for PRRS vaccine attenuation and reversion are discussed.

RNA recombination in Porcine Reproductive and Respiratory Syndrome Virus is restricted to parental sequences with high similarity

Two types of porcine reproductive and respiratory syndrome virus (PRRSV) exist, a North American type and a European type. The co-existence of both types in some countries, such as Denmark, Slovakia and Canada, creates a risk of inter-type recombination. To evaluate this risk, cell cultures were co-infected with either a North American and a European type of PRRSV or two diverse types of European isolate. Subsequently, an approximately 600 bp region of the PRRSV genome was tested for recombination by quantitative real-time RT–PCR. Between 0·1 and 2·5% RNA recombination was found between the European isolates, but no recombination was detected between the European and North American types. Calculation of the maximum theoretical risk of European–American recombination, based on the sensitivity of the RT–PCR system, revealed that RNA recombination between the European and North American types of PRRSV is at least 10000 times less likely to occur than RNA recombination between diverse European isolates.
Semen from boars infected with porcine reproductive and respiratory syndrome virus (PRRSV) contains antibodies against structural as well as nonstructural viral proteins

The seminal excretion of antibodies against porcine reproductive and respiratory syndrome virus (PRRSV) was examined in a group of five boars experimentally infected by the nasopharyngeal route. By using phage-displayed peptide epitopes from the PRRSV replicase and envelope glycoproteins as ELISA antigen, we were able to separately and specifically assay antibody responses against structural and nonstructural viral proteins. Antibodies against structural as well as nonstructural viral proteins were consistently found in the semen of all boars, beginning from 1-4 weeks postinfection. This is the first report documenting the presence of anti-PRRSV antibodies in boar semen. Seminal antiviral IgA was also detected, and we observed a correlation between seminal IgA responses against nonstructural viral proteins, and the duration of PRRSV RNA excretion in semen. The implications of these findings for the diagnostics and pathogenesis of venereal PRRSV infection are discussed.

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Oleksiewicz, M. B., Bøtner, A., Normann, P.
Publication date: 2001
Published pages: 109-125
Peer-reviewed: Yes
Transplacental infection with PCV-2 associated with reproductive failure in a gilt

A longitudinal study of serological patterns of respiratory infections in nine infected Danish swine herds

Circovirus og PMWS – hvor står vi
Designing serological surveillance programmes to document freedom from disease with special reference to exotic viral diseases of pigs in Denmark

Surveillance programmes based on laboratory screening tests are increasingly used to document freedom from disease in order to facilitate trade. The following aspects must be considered when designing such programmes: diseases to be selected; epidemiology of the diseases; unit of analysis (animal or herd); target age group (or target farm type); test characteristics and sample size. Issues related to these aspects are discussed and illustrated using the example of serological surveillance for exotic viral diseases in the pig population of Denmark. Sampling designs based on individual animal samples are compared with herd-based sampling (two-stage sampling). While the latter is likely to require a larger sample size, the increased level of information and the reliability of the results obtained are considered to be worth the expense. Issues related to the development of international standards for declaring freedom from disease are discussed. The authors conclude that international standards are desirable, providing that these standards represent scientifically valid principles.

General information
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Organisations: Division of Microbiology and Risk Assessment, National Food Institute, Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Stark, K., Mortensen, S., Olsen, A., Barfod, K., Betner, A., Lavritsen, D., Strandbygaard, B.
Pages: 715-724
Publication date: 2000
Peer-reviewed: Yes
Diagnostik vedrørende porcint circovirus type 2

General information
Publication status: Published
Organisations: Sektion for Ekotsiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A.
Pages: 5-6
Publication date: 2000
Peer-reviewed: No

Publication information
Journal: SVS/SVIV Information
Volume: 71
Original language: Danish
Source: orbit
Source-ID: 241426
Research output: Contribution to journal › Journal article – Annual report year: 2000 › Research

Distinction between infections with European and American/vaccine type PRRS virus after vaccination with a modified-live PRRS virus vaccine

In July 1996 a modified live Porcine reproductive and respiratory syndrome (PRRS) vaccine, based on an American (US) strain of the PRRS virus (PRRSV), was licensed in Denmark. The vaccine was licensed for use in 3-18 week old pigs, exclusively. Starting during the middle of October 1996, several herds who had recently begun vaccination, experienced acute PRRS-like symptoms including an increasing number of abortions and stillborn piglets and an increasing mortality in the nursing period. During the period from October 1996 until May 1997, the PRRS virus (PRRSV), identified as the vaccine/US type of PRRSV, was isolated from fetuses, dead piglets, pleural fluids and/or lung tissues from 114 of such herds. These findings indicated the spread of the vaccine virus to non-vaccinated sows following by transplacental infection of fetuses. Also, a number of not previously PRRSV infected and non-vaccinated herds in Denmark have become infected with the vaccine-like PRRSV. Possible routes of transmission are the introduction of vaccinated pigs to the herd, use of semen from artificial insemination (AI) centres or airborne transmission. The situation of PRRS in Denmark is now complicated by the fact that both the European (EU) type and the US type of PRRSV are circulating among their herds. It is not clinically possible to differentiate between the two different types of infections. At the Danish Veterinary Institute for Virus Research (DVIVR), diagnostic tools have been developed to distinguish between the two types of infections, and both virological and serological methods are now available for distinction. The diagnostic tests used at DVIVR to diagnose PRRS and to differentiate between EU and US strains of PRRSV infections are described below. The distinction between infection with the two types of PRRSV was made on a serological basis. The immunoperoxidase monolayer assay (IPMA), carried out using a Danish strain (IPMA/DK) and the vaccine strain (IPMA/vac) in parallel, allows the distinction of infections with EU and US strains of PRRSV. In herds infected with the EU type, the titer in individual samples is higher in the IPMA/DK compared to the titer in the IPMA/vac, while in herds infected with the vaccine/US type, the titers are highest in the IPMA/vac. Furthermore, a double blocking ELISA has been developed, which enables large scale screening for and simultaneous distinction between antibodies against EU and US strains of PRRSV. This test is performed using a Danish PRRSV isolate and the vaccine strain in parallel. The results of the ELISA tests are given as negative or positive for each sample. For positive samples a ratio is calculated (ratio = ODp blocking ELISA-DK / ODp blocking ELISA-Vac), which enables us to serologically distinguish between EU and US strains of PRRSV infections. In herds infected with the Danish strain of PRRSV, most animals have a ratio below 1, while in herds infected with the vaccine/US strain most animals have a ratio above 2. The distinction between infections with the two types of PRRSV was made by virus isolation. As the porcine pulmonary alveolar macrophages (PPAM) are found to be the most sensitive system for isolation of PRRSV field strains, while the vaccine strain, when taken directly from the bottle, grows in the MARC 145 cell line and not in the PPAM, both cell types have routinely been used in parallel at our institute since the introduction of the PRRS vaccine in Denmark. However, from our experience, the vaccine virus becomes able to replicate in PPAM during in vivo passages. For typing of the PRRSV isolates as EU or US/vaccine isolates, three monoclonal antibodies (mAb) are used in an IPMA: SDOW 17 reacting with most EU and US isolates including the vaccine strain, VO 17 reacting with some US isolates including the vaccine strain but not with EU PRRSV isolates, and WBE 4 reacting with most EU isolates, but not with US isolates. The detection and typing of PRRSV was made by RT-PCR. An RT-PCR test which sensitively detects and types PRRSV from relevant biological material and provides a maximal amount of sequence information by amplification of whole viral open reading frames, has been developed at our institute. To provide maximal sequence information, complete viral open reading frames (ORFs 5 and 7) are targeted for amplification. Typing of viruses is accomplished by any one of three strategies: (a) DNA sequencing, (b) type-specific PCR primers, (c) size determination of ORF7 amplicons. All three typing strategies show complete concordance with the currently used method of typing with monoclonal antibodies when used on a panel of PRRSV field isolates covering the period 1992-1997. The ORF7-based test had particularly desirable characteristics, namely highly sensitive detection of PRRSV without apparent type bias, typing of the detected virus, discrimination between pure and mixed virus populations, and semiquantitative assessment of type-ratios in mixed
populations, all in a single PCR reaction. The blocking ELISA is routinely used at our institute for PRRSV antibody screening, making it possible to distinguish between the EU and US type PRRSV in infected herds. IPMA is mostly used for serologic herd profiles to determine the spread of PRRSV within herds, based on the level of the IPMA titers. However, the IPMA can also be used to differentiate between the two different PRRSV type of infections. For detection and typing of PRRSV, we routinely use virus isolation and mAb typing. For selected cases RT-PCR followed by DNA sequencing are performed to confirm mAb typing or get more detailed information.

**General information**
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Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A., Strandbygaard, B., Sørensen, K. J., Oleksiewicz, M. B., Storgaard, T.
Pages: 72-73
Publication date: 2000
Peer-reviewed: Yes

**Publication information**
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Web of Science (2000): Indexed yes
Original language: English
Source: orbit
Source-ID: 241016
Research output: Contribution to journal › Journal article – Annual report year: 2000 › Research › peer-review

Emergence of porcine reproductive and respiratory syndrome virus deletion mutants: Correlation with the porcine antibody response to a hypervariable site in the ORF 3 structural glycoprotein

By using porcine immune sera to select a library of phage-displayed random peptides, we identified an antigenic sequence (RKASLSTS) in the C-terminus of the ORF 3 structural glycoprotein of European-type porcine reproductive and respiratory syndrome virus (PRRSV). Through the use of overlapping reading frames, the same PRRSV genetic locus codes for the ORF 3 "RKASLSTS" sequence, and a previously described ORF 4 epitope (Meulenherg, J. J. M., Van Nieuwstadt, A. P., Van Essen-Zandbergen, A., and Langeveld, J. P. M., 1997, J. Virol. 71, 6061-6067). Sequence analysis identified naturally occurring deletion mutants at this ORF 3/4 site. Phylogenetic analysis showed the presence of a highly accurate ORF 3 molecular clock, according to which deletion mutants and nondeleted viruses evolved at differing speeds. Furthermore, deletion mutants and nondeleted viruses evolved as separate lineages. These distinctions suggested that deletion mutants were a hitherto unrecognized subtype of European-type PRRSV. Currently, deletion mutants appear to be outcompeting nondeleted viruses in the field, highlighting the importance of the porcine antibody response against the minor structural glycoproteins of European-type PRRSV for viral evolution.

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Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute, Stald/vægterservice
Contributors: Oleksiewicz, M., Bøtner, A., Toft, P., Grubbe, T., Nielsen, J., Kamstrup, S., Storgaard, T.
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Web of Science (2000): Indexed yes
Original language: English
Source: orbit
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Research output: Contribution to journal › Journal article – Annual report year: 2000 › Research › peer-review
Maximum likelihood and Gibbs sampling estimation of sensitivity and specificity of a blocking ELISA and two immune peroxidase assays for measuring antibodies against PRRS-virus

General information
Publication status: Published
Organisations: Division of Microbiology and Risk Assessment, National Food Institute, Section for Veterinary Epidemiology and public sector consultancy, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Sektion for Eksotiske Virussygdomme, Division of Virology
Contributors: Barfod, K., Enøe, C., Bøtner, A., Strandbygaard, B.
Publication date: 2000
Peer-reviewed: No
Event: Abstract from 16th International Pig Veterinary Society Congress, Melbourne, Australia.
Source: orbit
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Research output: Contribution to conference › Conference abstract for conference – Annual report year: 2000 › Research

Molekylærepidemiologisk udredning af PRRSV udbredet i Europa

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Storgaard, T., Oleksiewicz, M., Bøtner, A.
Publication date: 2000

Publication information
Publisher: SVS/SVIV
Original language: English

Bibliographical note
Årsberetning
Source: orbit
Source-ID: 242196
Research output: Book/Report › Report – Annual report year: 2000 › Research

Post-weaning multisystemic wasting syndrome: Besætninger med PMWS-symptomer søges

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute, Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research
Contributors: Hassing, A., Bøtner, A., Jorsal, S. E. L.
Publication date: 2000
Peer-reviewed: No

Publication information
Journal: DS Nyt
Volume: 2
Issue number: 12
Original language: English
Source: orbit
Source-ID: 242188
Research output: Contribution to journal › Journal article – Annual report year: 2000 › Research

Post-weaning Multisystemic Wasting Syndrome, PMWS

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute, Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research
Contributors: Bøtner, A., Jorsal, S. E. L., Hassing, A. G.
Pages: 2-3
Publication date: 2000
Peer-reviewed: No
Post-weaning Multisystemic Wasting Syndrome (PMWS) og circovirus type 2

General information
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Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A.
Publication date: 2000
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Transfer of pathogens from sows to offspring

General information
Publication status: Published
Organisations: Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Division of Microbiology and Risk Assessment, National Food Institute, Sektion for Eksotiske Virussygdomme, Division of Virology, Management, Secretariat
Contributors: Lavrsen, D. T., Angen, Ø., Barfod, K., Bøtner, A., Lohse, L., Møller, K., Nielsen, J., Sørensen, V., Vigre, H.
Publication date: 2000

A live PRRSV vaccine: Mutations associated with attenuation and virulence

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Storgaard, T., Oleksiewicz, M. B., Bøtner, A.
Number of pages: 51
Determination of 5' -leader sequences from radically disparate strains of porcine reproductive and respiratory syndrome virus reveals the presence of highly conserved sequence motifs

We determined the untranslated 5'-leader sequence for three different isolates of porcine reproductive and respiratory syndrome virus (PRRSV): pathogenic European- and American-types, as well as an American-type vaccine strain. 5'-leader from European- and American-type PRRSV differed in length (220 and 190 nt, respectively), and exhibited only approximately 50% nucleotide homology. Nevertheless, highly conserved areas were identified in the leader of all 3 PRRSV isolates, which constitute candidate motifs for binding of protein(s) involved in viral replication. These comparative data provide a priori knowledge for mutational identification of virulence determinants in the 5' nontranslated part of the PRRSV genome.

Determination of 5'-leader sequences of different PRRSV strains

Diagnosing vaccine induced PRRS: Distinction between infections with European and American/vaccine type PRRS virus after vaccination with a modified-live PRRS virus vaccine

Diagnosing vaccine induced PRRS: Distinction between infections with European and American/vaccine type PRRS virus after vaccination with a modified-live PRRS virus vaccine
Examination of the selective pressures on a live PRRS vaccine virus

We determined the ORF5 and 7 sequences of 20 pathogenic revertants of a live PRRSV vaccine. The sequence analysis confirmed all 20 isolates to be of vaccine origin. Having established that clonal introduction of American (vaccine) PRRS virus had occurred in Denmark, we could perform analysis of the selective pressure this attenuated virus had experienced during reversion. An analysis of nucleotide mutations showed a similar rate of mutations in the two genes (ORF5 and 7). However, non-synonymous mutations in ORF7 were eliminated by purifying selection. In contrast, non-synonymous mutations in ORF5 were tolerated or even selected for. The cDNA sequencing of the 20 vaccine virus revertants identified two single nucleotide mutations located in ORF5 and in ORF6 that we suggest are involved or at least linked to the attenuation of the vaccine virus and to the subsequent reversion to virulence.

Heterologous challenge with porcine reproductive and respiratory syndrome (PRRS) vaccine virus: no evidence of reactivation of previous European-type PRRS virus infection

In Denmark, a porcine reproductive and respiratory syndrome virus (PRRSV) control programme, comprising vaccination of seropositive herds with a live American type PRRSV vaccine, was started in 1996. In several of these herds, spread of vaccine virus from vaccinated 3-18 week old pigs to non-vaccinated sows was demonstrated by the isolation of vaccine virus from fetuses and stillborn piglets. Surprisingly, sows infected with the American type vaccine strain consistently exhibited significantly stronger serological responses towards European type PRRSV than American type PRRSV. To order to elucidate whether the unexpectedly strong serological reaction towards European-type PRRSV in American type PRRSV infected sows was due to a booster reaction, or reactivation of an unrecognized, latent infection in the sows with European type PRRSV, a challenge study with the vaccine was carried out. In this study, the stronger serological response towards European type PRRSV than towards American type PRRSV was reproduced, and reactivation of the previous natural infection with European PRRSV could neither be demonstrated by virus isolation nor by RT-PCR. So, the increase in antibody titers towards European PRRSV in previously European PRRSV infected pigs after challenge with the vaccine strain seems to be the result of a boosting effect on the immune system, induced by the heterologous vaccine PRRSV strain.
Isolation of hæmagglutinerende encephalomyelitis virus fra pattegrise

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A.
Pages: 5-6
Publication date: 1999
Peer-reviewed: No

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Journal: SVS/SVIV Information
Volume: 64
Original language: Danish
Source: orbit
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Research output: Contribution to journal › Journal article – Annual report year: 1999 › Research

Isolation and characterisation of circoviruses from pigs with wasting syndromes in Spain, Denmark and Northern Ireland

A porcine circovirus (PCV) was isolated from tissues of pigs with wasting syndromes from Spain, Denmark and N. Ireland. The antigenic profiles of these viruses were determined by indirect immunofluorescence assays using polyclonal antisera and monoclonal antibodies (mAbs) prepared against previously isolated PCVs. A rapid and convenient PCR-based test was developed and used for the genotyping of these PCV isolates. These PCV isolates were found to be antigenically and genomically similar to previously reported isolates of PCV from pigs with wasting disease (PCV2), but distinct from the isolate of PCV from continuous PK/15 cell cultures (PCV1).

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Pages: 115-123
Publication date: 1999
Peer-reviewed: Yes

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Journal: Veterinary Microbiology
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Source: orbit
Monitoring of PRRSV infection status in swine herds based on analysis of antibodies in meat sample drippings

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- Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
- Contributors: Mortensen, S., Strandbygaard, B., Bøtner, A., Feld, N., Willeberg, P.
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- Publication date: 1999

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PCR til påvisning af PRRS-virus

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- Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
- Contributors: Bøtner, A., Oleksiewicz, M., Storgaard, T.
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- Original language: Danish
- Source: orbit
- Source-ID: 241416

PMWS – en ny svinesygdom

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- Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
- Contributors: Bøtner, A.
- Publication date: 1999
- Peer-reviewed: No
- Event: Abstract from Kongres for Svineproducenter, Herning.
- Source: orbit
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Porcin reproduktions- og respirations sygdom (PRRS) - en oversigtsartikel med fokus på den danske situation og erfaringer med anvendelsen af en levende vaccine

**General information**
- Publication status: Published
- Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
- Contributors: Bøtner, A., Toft, P. S.
- Pages: 175-182
- Publication date: 1999
- Peer-reviewed: No

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PRRS overvågning på kædsafprøver fra svinebesætninger

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Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Mortensen, S., Strandbygaard, B., Feld, N., Bøtner, A., Willeberg, P.
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Status vedrørende circovirus infektioner i Danmark

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Peer-reviewed: No
Event: Abstract from DVHS, .
Source: orbit
Source-ID: 242187
Research output: Contribution to conference › Conference abstract for conference – Annual report year: 1999 › Research

Virussygdomme hos svin. PPV, PRRS, svineinfluenza og porcint circovirus

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A.
Publication date: 1999

Publication information
Original language: English
Source: orbit
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Research output: Book/Report › Compendium/lecture notes – Annual report year: 1999 › Education

Blocking ELISA's for the distinction between antibodies against European and American strains of porcine reproductive and respiratory syndrome (PRRS) virus

A double blocking ELISA was developed in order to satisfy the need for large scale serological screening for PRRS and simultaneous distinction between infection with European and American strains of PRRSV in pig herds. The Immunoperoxidase monolayer assay (IPMA) and the double blocking ELISA enabled distinction on serological basis between infection with European and American strains of PRRSV. The distinction was possible from about day 7 after infection of pigs with PRRSV. The double blocking ELISA enabled the distinction at later stages of infection compared to the IPMA, irrespective of the strain involved.
Blocking ELISA's for the distinction between antibodies against European and American strains of porcine reproductive and respiratory syndrome virus

A double blocking ELISA was developed in order to satisfy the need for large scale serological screening for PRRS and simultaneous distinction between infection with European and American strains of PRRSV in pig herds. The Immunoperoxidase monolayer assay (IPMA) and the double blocking ELISA enabled distinction on serological basis between infection with European and American strains of PRRSV. The distinction was possible from about day 7 after infection of pigs with PRRSV. The double blocking ELISA enabled the distinction at later stages of infection compared to the IPMA, irrespective of the strain involved.

Challenge of previously PRRS infected pigs with PRRS vaccine virus

The Proceedings of the 15th International Pig Veterinary Society Congress

The Proceedings of the 15th International Pig Veterinary Society Congress
Epidemiology of PRRS in Danish pig herds

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Nymark, K., Pedersen, P. N., Thorup, F., Bækbo, P., Bøtner, A.
Number of pages: 257
Publication date: 1998

Host publication information
Title of host publication: The Proceedings of the 15th International Pig Veterinary Society Congress
Volume: 2
Publisher: Nottingham University Press
ISBN (Print): 18-97-67684-0
Source: orbit
Source-ID: 241407

Erfaringer vedrørende Porcint circovirus i danske svinebesætninger og Piglet Wasting Disease eller Post-weaning Multisystemic Wasting Syndrome

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A.
Pages: 6-8
Publication date: 1998
Peer-reviewed: No

Publication information
Journal: SVS/SVIV Information
Volume: 61
Original language: Danish
Source: orbit
Source-ID: 241410
Research output: Contribution to journal – Journal article – Annual report year: 1998 – Research

Esperienza Danese sull’impiego di un vaccino PRRS vivo modificato nel contesto di un programma di controllo della PRRS - The Danish experience with use of a live modified PRRS vaccine in a PRRS control programme

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A.
Pages: 107-111
Publication date: 1998

Host publication information
Title of host publication: S.I.P.A.S. - Società Italiana di Patologia ed Allevamento dei Suini
Source: orbit
Source-ID: 241397
Research output: Chapter in Book/Report/Conference proceeding – Article in proceedings – Annual report year: 1998 – Research

Experimental inoculation of late-term pregnant sows with a field isolate of PRRS vaccine-like virus

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Experimental inoculation of swine at various stages of gestation with a Danish isolate of porcine reproductive and respiratory syndrome virus (PRRSV)

Following intranasal inoculation of three groups of pregnant swine (in total 11 dams) with a Danish isolate of porcine reproductive and respiratory syndrome virus (PRRSV) on or about day 85, 70 and 45 of gestation, respectively, reproductive disturbances were observed in the first two groups. Transplacental transmission of PRRSV occurred in four out of five litters from dams inoculated around day 85 of gestation and in two out of three litters from dams inoculated on day 72 of gestation. In the third group, inoculated around day 45 of gestation, transplacental infection could not be demonstrated. Thirty-two (56%) piglets from dams inoculated on day 85 of gestation and 14 (33%) piglets from dams inoculated on day 72 of gestation, were transplacentally infected. Sixteen (28%) and six (14%) piglets, respectively, in these groups became infected in the perinatal period. Thirty-two (56%) piglets from dams inoculated on day 85 of gestation were stillborn or died within a 6-8 weeks observation period, 29 being stillborn or dying within the first two weeks of observation. Thirteen (30%) piglets from dams inoculated on day 72 of gestation died within the two weeks observation period. The duration of the viraemic phase varied considerably, from one day to four weeks, for both dams and their offspring. Most frequently, PRRSV was isolated from lung and/or tonsill tissues from dead and euthanized piglets younger than 14 days of age. Histopathological investigations of piglets typically revealed focal nonsuppurative inflammatory conditions, especially in the lung and heart. In conclusion, the present results support the hypothesis, that PRRSV infection of dams late in pregnancy has the greatest likelihood of transplacental infection of fetuses.

Field experiences with PRRS and with the use of a live vaccine in Denmark

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Kranker, S., Nielsen, J., Bille-Hansen, V., Bøtner, A.
Pages: 21-31
Publication date: 1998
Peer-reviewed: Yes

Publication information
Journal: Veterinary Microbiology
Volume: 61
Issue number: 1-2
ISSN (Print): 0378-1135
Original language: English
Keywords: pig-viruses, porcine reproductive and respiratory syndrome virus, transplacental infection
DOI: 10.1016/S0378-1135(98)00176-X
Source: orbit
Source-ID: 231082
Research output: Contribution to journal › Journal article – Annual report year: 1998 › Research › peer-review
Mapping the antigenic structure of porcine parvovirus at the level of peptides

The antigenic structure of the capsid proteins of porcine parvovirus (PPV) was investigated. A total of nine linear epitopes were identified by Pepscan using porcine or rabbit anti-PPV antisera. No sites were identified with a panel of neutralising monoclonal antibodies (MAbs). All epitopes were located in the region corresponding to the major capsid protein VP2. Based on this information, and on analogy to other autonomous parvoviruses, 24 different peptides were synthesised, coupled to keyhole limpet haemocyanin (KLH) and used to immunise rabbits. Most antisera were able to bind viral protein. Only peptides from the N-terminal part of VP2 were able to induce virus-neutralising antibodies, although at low levels. A similar neutralising activity could be obtained in pigs. The exposure of the N-terminus was shown in full virions, both by immuno-electron microscopy and absorption experiments. It is concluded that in PPV, the VP2 N-terminus is involved in virus neutralisation (VN) and peptides from this region are therefore primary targets for developing peptide-based vaccines against this virus.

General information
Publication status: Published
Organisations: National Veterinary Institute, Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Pages: 163-173
Publication date: 1998
Peer-reviewed: Yes

Publication information
Journal: Virus Research
Volume: 53
Issue number: 2
ISSN (Print): 0168-1702
Original language: English
Keywords: porcine parvovirus, anti-peptide immunization, peptides, Pepscan, antigenic structure
DOIs:
10.1016/S0168-1702(97)00145-7
Source: orbit
Source-ID: 230328
Research output: Contribution to journal › Journal article – Annual report year: 1998 › Research › peer-review

Novel porcine circoviruses from diseased pigs with wasting disease syndromes

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Pages: 467-468
Publication date: 1998
Peer-reviewed: Yes

Publication information
Journal: Veterinary Record
ISSN (Print): 0042-4900
Original language: English
Source: orbit
Source-ID: 241412
Research output: Contribution to journal › Journal article – Annual report year: 1998 › Research › peer-review

Novel porcine circoviruses from pigs with wasting disease syndromes

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
PCR til påvisning og typning af PRRS-virus

Porcin reproduktions- og respirationssygdom (PRRS)

PRRS - the Danish experience, 1998
Rabies infection of European bats and their zoonotic significance

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Rønsholt, L., Bøtner, A.
Publication date: 1998
Peer-reviewed: No
Event: Abstract from The 2nd International Conference on Emerging Zoonoses, Strassburg, France.
Source: orbit

Sensitive detection and typing of porcine reproductive and respiratory syndrome virus by RT-PCR amplification of whole viral genes

Following the recent use of a live vaccine against porcine reproductive and respiratory syndrome virus (PRRSV) in Denmark, both American (vaccine) and European-type PRRSV now coexist in Danish herds. This situation highlighted a requirement for supplementary tests for precise virus-typing. As a result, we developed a RT-PCR assay able to detect as well as type PRRSV. To provide maximal sequence information, complete viral open reading frames (ORFs 5 and 7) were targeted for amplification. The RT-PCR test was able to amplify complete PRRSV ORFs from complex materials such as boar semen containing as little as 1 TCID50 ml⁻¹ of PRRSV. Typing of viruses was accomplished by any one of three strategies: (i) use of type-specific PCR primers, (ii) size determination of ORF 7 amplicons, (iii) DNA sequencing. All three typing strategies showed complete concordance with the currently used method of typing with monoclonal antibodies (MAbs) when used on a panel of PRRSV field isolates covering the period 1992-1997. The ORF 7-based test had particularly desirable characteristics, namely, highly sensitive detection of PRRSV without apparent type bias, typing of the detected virus, discrimination between pure and mixed virus populations, and semi-quantitative assessment of type ratios in mixed populations, all in a single PCR reaction. In addition, the obtained sequence data were used to predict two simple and rapid strategies (single-enzyme restriction length polymorphism analysis and oligonucleotide hybridization) for confirmation of the specificity of ORF 7 RT-PCR reactions. As such, the RT PCR assay provides a new, powerful diagnostic tool to study the population dynamics between present and emerging PRRSV-types.

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute, Stald/vægterservice
Contributors: Oleksiewicz, M., Bøtner, A., Madsen, K., Storgaard, T.
Pages: 7-22
Publication date: 1998
Peer-reviewed: Yes

Publication information
Journal: Veterinary Microbiology
Volume: 64
Issue number: 1
ISSN (Print): 0378-1135
Original language: English
Keywords: pig-viruses, PRRSV, RT-PCR, diagnosis-viruses, RFLP, vaccinations
DOIs: 10.1016/S0378-1135(98)00254-5
Source: orbit
Source-ID: 231016

Sequence analysis of porcine reproductive and respiratory syndrome virus of the American type collected from Danish swine herds

Vaccine-like viruses of American type of porcine reproductive and respiratory syndrome virus (PRRSV) were detected in serum samples by RT-PCR. The viruses were analysed by nucleotide sequencing of the genomic region encoding open reading frames 2 to 7. During the ongoing study of Danish isolates of PRRSV by means of nucleotide sequencing, RT-PCR reactions and subsequent nucleotide sequencing showed the presence of American type PRRSV in Danish breeding herds. Most likely, these atypical viruses originated from boars vaccinated with live vaccine of American type (MLV RespPRRS), which were taken to artificial insemination centres and there brought together with unvaccinated boars.

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Rønsholt, L., Bøtner, A.
Publication date: 1998
Peer-reviewed: No
Event: Abstract from The 2nd International Conference on Emerging Zoonoses, Strassburg, France
Source: orbit

Publication information
Journal: Veterinary Microbiology
Volume: 64
Issue number: 1
ISSN (Print): 0378-1135
Original language: English
Keywords: pig-viruses, PRRSV, RT-PCR, diagnosis-viruses, RFLP, vaccinations
DOIs: 10.1016/S0378-1135(98)00254-5
Source: orbit
Source-ID: 231016

Research output: Contribution to journal › Journal article – Annual report year: 1998 › Research › peer-review
already at the centres. The nucleotide sequences of three Danish viruses of American type PRRSV were compared to those of known PRRSV isolates. The nucleotide sequence identities of the atypical Danish isolates were between 99.2-99.5% to the vaccine virus RespPRRS and 99.0-99.3% to VR2332 which are the parental virus to the vaccine virus. Phylogenetic analysis including field isolates of American type supports the conclusion that the introduction of American type PRRSV in Denmark was due to spread of vaccine virus.

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Madsen, K., Hansen, C., Madsen, E., Strandbygaard, B., Bøtner, A., Sørensen, K.
Pages: 1683-1700
Publication date: 1998
Peer-reviewed: Yes

Publication information
Journal: Archives of Virology
Volume: 143
Issue number: 9
ISSN (Print): 0304-8608
Original language: English
Source: orbit
Source-ID: 231043
Research output: Contribution to journal › Journal article – Annual report year: 1998 › Research › peer-review

The Danish experience with use of a live modified PRRS vaccine in a PRRS control programme

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A.
Pages: 8-17
Publication date: 1998

Host publication information
Title of host publication: Proceedings of 3. Munich Symposium : Diseases of pig
Source: orbit
Source-ID: 242179
Research output: Chapter in Book/Report/Conference proceeding › Article in proceedings – Annual report year: 1998 › Research

A case-control questionnaire survey of risk factors for Porcine Reproductive and Respiratory Syndrome (PRRS) seropositivity in Danish swine herds

Sixty-eight case herds seropositive to porcine reproductive and respiratory syndrome (PRRS) were compared to 128 seronegative controls in a double-blinded questionnaire survey. The study indicated no increased risk of PRRS seropositivity for herds using artificial insemination with semen from PRRS seropositive AI-stations. Also the herd-size was non-related to the risk of PRRS seropositivity, indicating that air-borne spread of PRRS may not have been a predominant feature in Denmark. Introduction of replacement breeding animals from seropositive breeding- and multiplying herds significantly increased the risk of a herd being PRRS seropositive, as did introduction of 25 kg pigs for feeding. PRRS seropositivity was in the farmers’ opinions associated with abortions in sows, early farrowing, high postweaning mortality and low weight gain in fattening pigs. However, the reported frequencies of problems were relatively low.

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Mousing, J., Permin, A., Mortensen, S., Bøtner, A., Willeberg, P.
Pages: 323-328
Publication date: 1997
Peer-reviewed: Yes

Publication information
Journal: Veterinary Microbiology
Volume: 55
Issue number: 1-4
ISSN (Print): 0378-1135
Original language: English
Diagnosis of PRRS

This paper reviews various diagnostic methods for the detection of porcine reproductive and respiratory syndrome (PRRS) virus or antibodies to PRRS virus reported during the period from 1991 to 1995. In addition, experience from a European Community Concerted Action and especially Danish experiences concerning serological tests are presented. It is concluded that, in general, serological diagnosis with a high specificity and sensitivity is easy to perform on a herd level. However, no serological test has proven to be suitable for individual animal certification.

General information
A blocking ELISA was developed for the detection of antibodies against PRRS virus with a view to satisfying the need for examination of blood samples on a large scale. The test was evaluated in comparison with an indirect Elisa and the immunoperoxidase monolayer assay. The blocking Elisa was sensitive and specific. It had a higher capacity and was cheaper to perform than the immunoperoxidase monolayer assay and the indirect Elisa. It was comparable to the immunoperoxidase monolayer assay and better than the indirect Elisa in detecting antibodies formed early after infection, and it was superior to both the immunoperoxidase monolayer assay and the indirect Elisa in detecting antibodies at a late stage of infection.
Examination of virus shedding in semen from vaccinated and from previously infected boars after experimental challenge with porcine reproductive and respiratory syndrome virus

Danish artificial insemination (AI) centres house several boars antibody positive to porcine reproductive and respiratory syndrome virus as well as PRRSV-naive boars which may become acutely infected. The risk of transmission of PRRSV by semen may therefore constitute a serious problem to the Danish pig industry. The use of a vaccination-program may be a way to avoid or reduce the problem. This study evaluates the use of two vaccines: One live, attenuated vaccine and one inactivated vaccine. A pronounced reduction in viremia and shedding of virus in semen was demonstrated by use of the live vaccine compared to the non-vaccinated control animals. In contrast, no changes in onset, level and duration of viremia and shedding of virus in semen were observed using the inactivated vaccine. Neither viremia nor seminal shedding of virus was detected in previously PRRSV-infected, PRRSV-antibody positive boars after challenge with a Danish field strain of PRRSV.

Hematological and immunological parameters of 4-1/2-month-old pigs infected with PRRS virus

4-1/2-month old SPF pigs were experimentally infected with PRRS virus. Blood samples were collected with regular intervals up to day 35 post inoculation (PI). Serum was used for PRRS virus isolation and antibody detection and stabilized blood for total leucocyte counts, differential counts and characterization of lymphocyte subpopulations by flow cytometry analysis using monoclonal antibodies specific for porcine CD2, CD4 and CD8. After an initial viremic period of 1–7 days duration for individual pigs, PRRS virus was intermittently detected in pigs up to day 18 PI. All pigs had developed antibodies against PRRS virus by day 14 PI. Total blood leucocyte counts and lymphocyte counts were significantly decreased for a few days shortly after infection, but had returned to pre-infection levels on day 8–10 PI. A major change in the distribution of lymphocyte subpopulations was observed on day 3 PI, where the percentages of CD2+, CD4+ and CD8+ cells were significantly decreased. However, the percentages of these lymphocyte subsets quickly returned to approximately pre-infection values. The observed changes of the parameters examined do not indicate long-term systemic immunosuppression of the infected pigs.
Serologiske besætningsprofiler: PRRS, influenza. Pfizer symposium, Kontrol af luftvejslidelser hos svin i fremtidens produktionssystemer

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A.
Pages: 13-15
Publication date: 1997
Peer-reviewed: No

Publication information
Journal: DANSK VETERINÆRTIDSSKRIFT
Volume: Særtryk
ISSN (Print): 0106-6854
Original language: Danish
Source: orbit
Source-ID: 241387
Research output: Contribution to journal › Journal article – Annual report year: 1997 › Research

Spread of PRRS vaccine virus to non-vaccination swine herds in Denmark

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Strandbygaard, B., Bøtner, A., Sørensen, K. J., Have, P., Madsen, K. G., Madsen, E. S., Alexandersen, S.
Publication date: 1997
Peer-reviewed: No
Event: Poster session presented at 4th International Congress of Veterinary Virology, Edinburgh, United Kingdom.
Source: orbit
Source-ID: 241341
Research output: Contribution to conference › Poster – Annual report year: 1997 › Research

Spread of PRRS vaccine virus to non-vaccination swine herds in Denmark

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Strandbygaard, B., Bøtner, A., Sørensen, K. J., Have, P., Madsen, K. G., Madsen, E. S., Alexandersen, S.
Publication date: 1997
Peer-reviewed: No
Event: Abstract from 4th International Congress of Veterinary Virology, Edinburgh, United Kingdom.
Source: orbit
Source-ID: 241393
Research output: Contribution to conference › Conference abstract for conference – Annual report year: 1997 › Research

Vaccination mod PRRS i sobesætninger

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Nymark, K., Pedersen, P., Bøtner, A.
Pages: 9707
Varighed af immunitet i orner efter vaccination med en levende attenueret PRRS-virus vaccine

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Kranker, S., Bøtner, A.
Publication date: 1997

Publication information
Original language: Danish

Bibliographical note
Rapport til Danske Slagterier
Source: orbit
Source-ID: 241687
Research output: Book/Report › Report – Annual report year: 1997 › Research

Examination of virus shedding in semen from vaccinated and previously infected boars after experimental challenge with Porcine Reproductive and Respiratory Syndrome virus

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A., Nielsen, J., Have, P., Bækbo, P., Hoff-Jørgensen, R., Nielsen, T. L.
Publication date: 1996
Peer-reviewed: Yes
Event: Abstract from Eight European A.I. Vets Meeting, Billund, Denmark, .
Source: orbit
Source-ID: 241686
Research output: Contribution to conference › Conference abstract for conference – Annual report year: 1996 › Research › peer-review

PRRS-serologi

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A.
Publication date: 1996
Peer-reviewed: No

Publication information
Journal: Veterinaerfaglige møder om PRRS og salmonella
Original language: Danish
Source: orbit
Source-ID: 242161
Research output: Contribution to journal › Conference abstract in journal – Annual report year: 1996 › Research

PRRS-serologi og erfaringer med PRRS vaccination i Danmark

General information
Publication status: Published
Spredning af PRRS-vaccine-virus

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Nielsen, J., Bøtner, A.
Pages: 22-24
Publication date: 1996
Peer-reviewed: No

Publication information
Journal: VeterinærInformation
Volume: 3
ISSN (Print): 0906-253X
Original language: Danish
Source: orbit
Source-ID: 241378
Research output: Contribution to journal › Journal article – Annual report year: 1996 › Research

Spredning af PRRS-vaccine-virus fra vaccinerede til uvaccinerede grise

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A., Nielsen, J.
Publication date: 1996
Peer-reviewed: No

Publication information
Journal: SVS/SVIV Information
Original language: Danish
Source: orbit
Source-ID: 241756
Research output: Contribution to journal › Journal article – Annual report year: 1996 › Research

Undersøgelse af sæd for dansk PRRS-virus og PRRS-vaccine-virus

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Mortensen, S., Madsen, K. S., Bøtner, A.
Pages: 12-14
Publication date: 1996
Peer-reviewed: No

Publication information
Journal: VeterinærInformation
Volume: 3
ISSN (Print): 0906-253X
Original language: English
Source: orbit
Source-ID: 241379
Research output: Contribution to journal › Journal article – Annual report year: 1996 › Research

Undersøgelse for udskillelse af PRRS-virus i sæd fra vaccinerede og tidligere inficerede, seropositive orner efter eksperimentel smitte med et dansk PRRS-virusisolat

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Nielsen, T. L., Kranker, S., Nielsen, J., Have, P., Hoff-Jørgensen, R., Bækbo, P., Bøtner, A.
Pages: 851-856
Publication date: 1996
Peer-reviewed: Yes
Analysis of a portion of the mitochondrial gene for the large subunit ribosomal RNA from Pneumocystis carinii isolated from Danish pigs

General information
Publication status: Published
Organisations: Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Sektion for Eksotiske Virussygdomme, Division of Virology
Publication date: 1995
Peer-reviewed: No
Event: Abstract from Second general meeting of the EU concerted action on Pneumocystis and Pneumocystosis, Madrid, Spain.
Source: orbit
Source-ID: 241750
Research output: Contribution to conference › Conference abstract for conference – Annual report year: 1995 › Research › peer-review

Examination of PRRS-virus-shedding in semen from vaccinated and non-vaccinated boars

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Publication date: 1995
Peer-reviewed: No
Event: Abstract from the 2nd International Symposium on Porcine Reproductive and Respiratory Syndrome (PRRS), Copenhagen.
Source: orbit
Source-ID: 241373
Research output: Contribution to conference › Conference abstract for conference – Annual report year: 1995 › Research

Influence of parental MHC class I on survival of offspring from sows naturally infected with PRRS-virus

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Kristensen, B., Nielsen, J., Bøtner, A.
Publication date: 1995
Ny ELISA til påvisning af antistof mod PRRS-virus

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A.
Publication date: 1995
Peer-reviewed: No

Publication information
Journal: SVS/SVIV Information
Original language: Danish
Source: orbit
Source-ID: 241754
Research output: Contribution to journal › Journal article – Annual report year: 1995 › Research

PRRS

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A.
Publication date: 1995
Number of pages: 55

Publication information
Publisher: SVS/SVIV
Original language: Danish
Source: orbit
Source-ID: 242154
Research output: Book/Report › Report – Annual report year: 1995 › Research

PRRS-spredning har taget fart i Danmark

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Mortensen, S., Bøtner, A.
Pages: 13-15
Publication date: 1995
Peer-reviewed: No

Publication information
Journal: Veterinaire information
Volume: Ekstranummer
ISSN (Print): 0906-253X
Original language: Danish
Source: orbit
Source-ID: 241375
Research output: Contribution to journal › Journal article – Annual report year: 1995 › Research

Serologiske undersøgelser for PRRS

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Undersøgelse af RespPRRS-vaccine i orner. Udskillelse af PRRS-virus i sæd

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A., Nielsen, T.
Publication date: 1995

Publication information
Original language: Danish

Bibliographical note
Rapport til Boehringer Ingelheim
Source: orbit
Source-ID: 241683
Research output: Book/Report › Report – Annual report year: 1995 › Research

Undersøgelse for forekomst af PRRS-virus i ornesæd fra vaccinerede og ikke vaccinerede samt tidligere PRRS-inficerede, seropositive orner efter challenge med et dansk PRRS-virus isolat

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A., Nielsen, T. L.
Publication date: 1995

Publication information
Original language: Danish

Bibliographical note
Rapport til Danske Slagterier
Source: orbit
Source-ID: 241682
Research output: Book/Report › Report – Annual report year: 1995 › Research

Challenge study with swine influenza virus subtype H1N1 (A/Denmark/19126/93) after vaccination with swine influenza vaccine Derflu

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Have, P., Bøtner, A.
Publication date: 1994

Publication information
Original language: English

Bibliographical note
Rapport til Rhône Mérieux
Source: orbit
Source-ID: 241673
Research output: Book/Report › Report – Annual report year: 1994 › Research
Challenge study with swine influenza virus subtype H1N1 (A/Denmark/19126/93) after vaccination with swine influenza vaccine Griporiffa

**General information**
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Have, P., Bøtner, A.
Publication date: 1994

**Publication information**
Original language: English

**Bibliographical note**
Rapport til Rhône Mérieux
Source: orbit
Source-ID: 241676
Research output: Book/Report › Report – Annual report year: 1994 › Research

Challenge study with swine influenza virus subtype H3N2 (A/Denmark/15027/90) after vaccination with swine influenza vaccine Derflu

**General information**
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Have, P., Bøtner, A.
Publication date: 1994

**Publication information**
Original language: English

**Bibliographical note**
Rapport til Rhône Mérieux
Source: orbit
Source-ID: 241674
Research output: Book/Report › Report – Annual report year: 1994 › Research

Danske erfaringer med serologiske undersøgelser for PRRS og nyt vedrørende PRRS vacciner

**General information**
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A.
Pages: 86-91
Publication date: 1994
Peer-reviewed: No

**Publication information**
Journal: DVHS
Original language: Danish
Source: orbit
Source-ID: 242150
Research output: Contribution to journal › Conference abstract in journal – Annual report year: 1994 › Research

Diagnostik vedrørende PRRS

**General information**
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A., Mortensen, S.
Pages: 33-35
Publication date: 1994
Peer-reviewed: Yes
En undersøgelse af kliniske symptomer, serologiske profiler, produktions- samt økonomisk betydning af PRRS i klinisk udbrud i sønderjyske besætninger: Projekt "Syd"

Experimental infection of minipigs with porcine reproductive and respiratory syndrome (PRRS) virus

Isolation of porcine reproductive and respiratory syndrome (PRRS) virus in a Danish swine herd and experimental infection of pregnant gilts with the virus
Large scale screening for PRRS in Denmark

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A., Nielsen, J.
Publication date: 1994
Peer-reviewed: No
Event: Abstract from Fourth meeting of the concerted action on PRRS, CNEVA-Ploufragan, France, .
Source: orbit
Source-ID: 240999
Research output: Contribution to journal – Journal article – Annual report year: 1994 – Research

Påvisning af antistof mod PRRS-virus

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A.
Number of pages: 46
Publication date: 1994

Publication information
Publisher: SVS/SVIV
Original language: Danish

Bibliographical note
Årsberetning
Source: orbit
Source-ID: 241679
Research output: Contribution to conference – Conference abstract for conference – Annual report year: 1994 – Research

PRRS i Danmark - Sønderjylland er hårdest ramt

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A.
Publication date: 1994
Peer-reviewed: No

Publication information
Journal: Hyologisk
Volume: 2
Issue number: 8
ISSN (Print): 0906-0995
Original language: Danish
Source: orbit
Source-ID: 241746
Research output: Contribution to journal – Journal article – Annual report year: 1994 – Research

Svineinfluenza
Udbredelsen af PRRS i Danmark

Virologiske risici ved anvendelse af husdyrgødning og affald

Aujeszky's sygdom: Epizootiologiske og meteorologiske forhold i vintrene 1990/91 og 1991/92
Further evidence of long distance airborne transmission of Aujeszky's disease (pseudorabies) virus
In spite of the eradication of Aujeszky's disease in Denmark a single outbreak was recorded in December 1988 and another severe epizootic took place during the winter and spring of 1989/90. The epizootic occurred in nearly the same areas as the preceding epizootic during the winter of 1987/88. Identification of the strains of virus involved eliminated the possibility that the latest epizootic was due to the persistence of virus in the pig population. Furthermore, as during the preceding epizootic, initial recordings of the new strains were found to coincide with periods with southerly winds. It was concluded from circumstantial evidence that the concurrent introductions of virus to several farms played a major role during the epizootic.

Porcine Reproductive and Respiratory Syndrome (PRRS) in Denmark

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute, Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research
Contributors: Bøtner, A., Nielsen, J., Bille-Hansen, V.
Publication date: 1993

Production of porcine parvovirus empty capsids in a recombinant baculovirus/insect cell system. A candidate vaccine

General information
Publication status: Published
Organisations: National Veterinary Institute, Sektion for Ekspotiske Virussygdomme, Division of Virology
Contributors: Kamstrup, S., Casal, J. I., Meloen, R., Nielsen, J., Bøtner, A., Rønsholt, L., Have, P., Jensen, M. H., Dalsgaard, K.
Publication date: 1993
Peer-reviewed: No
Event: Poster session presented at 13th Nordic virus symposium, Copenhagen.
Source: orbit
Source-ID: 241338
Research output: Contribution to conference › Poster – Annual report year: 1993 › Research

Status vedrørende PRRS

General information
Publication status: Published
Organisations: Sektion for Ekspotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A.
Publication date: 1993
Peer-reviewed: No

Publication information
Journal: DVHS
Issue number: 18-21
Original language: Danish
Source: orbit
Source-ID: 242146
Research output: Contribution to journal › Conference abstract in journal – Annual report year: 1993 › Research

Virusinfektioner som årsag til reproduktions-förstyrrelser hos svin - herunder serologiske aspekter

General information
Publication status: Published
Organisations: Sektion for Ekspotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A.
Publication date: 1993

Host publication information
Title of host publication: Immunologi og Reproduktion
Publisher: Nordisk Veterinærforening for Husdyrreproduktion
Source: orbit
Source-ID: 241671
Research output: Chapter in Book/Report/Conference proceeding › Article in proceedings – Annual report year: 1993 › Research

Virus og diagnostik af virusinfektioner

General information
Publication status: Published
Organisations: Sektion for Ekspotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A.
Publication date: 1993
Peer-reviewed: No

Publication information
Virus screening of a bovine in vitro embryo production system

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Avery, B., Greve, T., Rønsholt, L., Bøtner, A.
Pages: 660
Publication date: 1993
Peer-reviewed: Yes

Publication information
Journal: Veterinary Record
Volume: 132
ISSN (Print): 0042-4900
Original language: English
Source: orbit
Source-ID: 240994
Research output: Contribution to journal › Journal article – Annual report year: 1993 › Research › peer-review

Porcin reproduktions- og respirationssygdom (PRRS)

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A., Nielsen, J.
Number of pages: 43
Publication date: 1992

Publication information
Publisher: SVS/SVIV
Original language: Danish

Bibliographical note
Årsberetning
Source: orbit
Source-ID: 242145
Research output: Book/Report › Report – Annual report year: 1992 › Research

Porcin Reproduktions- og Respirationssygdom virus

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A., Nielsen, J.
Publication date: 1992
Peer-reviewed: No
Event: Poster session presented at Den danske Dyrlægeforenings årskursus, Askov.
Source: orbit
Source-ID: 241337
Research output: Contribution to conference › Poster – Annual report year: 1992 › Research

Aujeszyk’s sygdom: En epizootiologisk vurdering af udbrud¬dene i perioden december 1989 - juli 1990

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Christensen, L. S., Mortensen, S., Bøtner, A., Rønsholt, L., Strandbygaard, S. B., Henriksen, C. A., Andersen, A. B., Andersen, J. B.
Aujeszky's sygdom: En epizootiologisk vurdering af udbruddene i perioden december 1989 - juli 1990

General information
Publication status: Published
Organisations: National Veterinary Institute, Sektion for Eksotiske Virussygdomme, Division of Virology
Contributors: Christensen, L. S., Mortensen, S., Bøtner, A., Rønsholt, L., Strandbygaard, B., Henriksen, C. A., Andersen, A. B., Andersen, J. B.
Pages: 253-255
Publication date: 1991
Peer-reviewed: No

Inactivation of Aujeszky's disease virus in slurry at various temperatures
Survival of Aujeszky's disease virus in pig slurry was investigated during anaerobic storage at 5, 20, 35, 40, 45, 50 and 55°C using 100-ml laboratory models simulating the conditions in slurry tanks during winter and summer seasons and during anaerobic digestion in batch reactors. The inactivation rate was found to increase with increasing temperature. Virus was inactivated at 5 and 20°C in 15 weeks and 2 weeks, respectively. At 35°C (mesophilic conditions) the virus was inactivated in 5 hours and at 55°C (thermophilic conditions) no virus could be detected after 10 minutes.

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A.
Pages: 225-235
Publication date: 1991
Peer-reviewed: Yes

The seal death i Danish waters. 2. Virological studies

General information
Modelstudier vedrørende overlevelse af virus i gylle under traditionel opbevaring og under udrådning i biogasanlæg

**General information**
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A.
Publication date: 1990

**Publication information**
Place of publication: Lindholm
Publisher: Statens Veterinære institut for virusforskning
Original language: Danish
Source: orbit
Source-ID: 241352
Research output: Book/Report → Report – Annual report year: 1990 → Research

Infection studies in mink with seal-derived morbillivirus

Morbillivirus derived from diseased harbour seals (Phoca vitulina) has characteristics of acute virulent canine distemper virus infection in mink. The infection induced a disease resembling the acute systemic and nervous form of canine distemper.

**General information**
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Blixenkrone-Møller, M., Svansson, V., Have, P., Bøtner, A., Nielsen, J.
Pages: 165-170
Publication date: 1989
Peer-reviewed: Yes

**Publication information**
Journal: Archives of Virology
Volume: 106
Issue number: 1-2
ISSN (Print): 0304-8608
Original language: English
DOI:
10.1007/BF01311049
Source: orbit
Source-ID: 240987
Research output: Contribution to journal → Journal article – Annual report year: 1989 → Research → peer-review

Virological studies of seal death in Denmark

**General information**
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Have, P., Nielsen, J., Bøtner, A., Moving, V.
Publication date: 1989
Bovine rota- og coronavirus. Specielt laboratorie-diagnostik og adaptering af virus til cellekulturer

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A.
Publication date: 1984

Bibliographical note
Licentiatafhandling ved KVL

Examination of rotavirus from bovine and porcine species by Western blotting

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bøtner, A., Askaa, J.
Number of pages: 222
Publication date: 1984

Host publication information
Title of host publication: Recent advances in virus diagnosis
Publisher: Springer
Editors: McNulty, M., McFerran, J.
ISBN (Print): 08-98-38674-8
Source-ID: 241669

Studies on the growth of bovine rotavirus in cell cultures

General information
Publication status: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Butchaiah, G., Bøtner, A., Lund, E.
Pages: 760-769
Publication date: 1984
Peer-reviewed: Yes

Publication information
Journal: ZENTRALBLATT FUR VETERINARMEDIZIN REIHE B-JOURNAL OF VETERINARY MEDICINE SERIES B-INFECTIOUS DISEASES IMMUNOLOGY FOOD HYGIENE VETERINARY PUBLIC HEALTH
Volume: 31
Original language: English
Source-ID: 240984

An outbreak of excessive neonatal mortality in four Danish mink farms. I. Descriptive epidemiological investigations
An outbreak of excessive neonatal mortality in four Danish mink farms. II. Analytic epidemiological investigations