Bacterial species associated with interdigital phlegmon outbreaks in Finnish dairy herds

Background: Severe outbreaks of bovine interdigital phlegmon (IP) have occurred recently in several free stall dairy herds in Finland. We studied the aetiology of IP in such herds, and the association of bacterial species with the various stages of IP and herds of various morbidity of IP. Nineteen free stall dairy herds with IP outbreaks and three control herds were visited and bacteriological samples collected from cows suffering from IP (n = 106), other hoof diseases (n = 58), and control cows (n = 64). The herds were divided into high morbidity (morbidity ≥50%) and moderate morbidity groups (9-33%) based on morbidity during the first two months of the outbreak. Results: F. necrophorum subspecies necrophorum was clearly associated with IP in general, and T. pyogenes was associated with the healing stage of IP. Six other major hoof pathogens were detected; Dichelobacter nodosus, Porphyromonas levii, Prevotella melaninogenica, Treponema spp. and Trueperella pyogenes. Most of the samples of acute IP (66.7%) harboured both F. necrophorum and D. nodosus. We found differences between moderate morbidity and high morbidity herds. D. nodosus was more common in IP lesion in high than in moderate morbidity herds. Conclusions: Our result confirms that F. necrophorum subspecies necrophorum is the main pathogen in IP, but also T. pyogenes is associated with the healing stage of IP. Our results suggest that D. nodosus may play a role in the severity of the outbreak of IP, but further research is needed to establish other bacteriological factors behind these severe outbreaks.
Screening for multiple tick-borne pathogens in Ixodes ricinus ticks from birds in Denmark during spring and autumn migration seasons

Presently, it is uncertain to what extent seasonal migrating birds contribute to the introduction of ticks and tick-associated pathogens in Denmark. To quantify this phenomenon, we captured birds during the spring and autumn migration at three field sites in Denmark and screened them for ticks. Bird-derived ticks were identified to tick species and screened for 37 tick-borne pathogens using real-time PCR. Overall, 807 birds, representing 44 bird species, were captured and examined for ticks during the spring (292 birds) and autumn migrations (515 birds). 10.7% of the birds harboured a total of 179 Ixodes ricinus ticks (38 ticks in spring and 141 in the autumn) with a mean infestation intensity of 2.1 ticks per bird. The European robin (Erithacus rubecula), the common blackbird (Turdus merula), and the common redstart (Phoenicurus
phoenicurus) had the highest infestation intensities. 60.9% of the ticks were PCR-positive for at least one tick-borne pathogen. Borrelia DNA was found in 36.9% of the ticks. The Borrelia species detected were B. spielmanii (15.1%), B. valaisiana (13.4%), B. garinii (12.3%), B. burgdorferi s.s. (2.2%), B. miyamotoi (1.1%), and B. afzelii (0.6%). In addition, 10.6% and 1.7% of the samples were PCR-positive for spotted fever group rickettsiae and Candidatus Neoehrlichia mikurensis. All of the tick-borne pathogens that we found in the present study are known to occur in Danish forest populations of I. ricinus. Our study indicates that migrating birds can transport ticks and their pathogens from neighboring countries to Denmark including sites in Denmark without a sustainable tick population. Thus, a tick-borne pathogen affecting human or animal health emerging at one location in Europe can rapidly be introduced to other countries by migrating birds. These movements are beyond national veterinary control. The current globalization, climatic and environmental changes affect the potential for introduction and establishment of ticks and tick-borne pathogens in Northern Europe. It is therefore important to quantify the risk for rapid spread and long distance exchange of tick-borne pathogens in Europe.

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Organisations: Bacteriology & Parasitology, National Veterinary Institute, Epidemiology, University of Copenhagen, Technical University of Denmark
Contributors: Klitgaard Schou, K., Højgaard, J., Isbrand, A., Madsen, J. J., Thorup, K., Bødker, R.
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Web of Science (2012): Impact factor 2.353
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Migrating birds and carnivores introduce ticks and tick-borne pathogens to Denmark – but are they also a public health risk?
Since the end of the ice age, spring migrating birds from Africa and Europe and autumn migrating birds from Northern Scandinavia have entered Denmark, and recently a small wave of long migrating carnivores have started arriving in
Denmark from Central Europe. Theoretically, migrating birds could introduce new tick species as well as tick-associated pathogens to Denmark. These migrating animals may also carry ticks and pathogens which already exist in native tick populations in Denmark. The potential supplement of native ticks and existing pathogens to the established high density tick populations in Danish forest and nature areas can be expected to be of little practical importance. However, some of the infected ticks, introduced by migrating birds, may be deposited in private gardens and public parks that are otherwise not able to sustain a viable tick population. Migrating birds may therefore introduce a low level risk of tick borne infections to urban areas. Also the recent unexpected wave of long migrating golden jackals (Canis aureus) and grey wolves (Canis lupus), arriving at the Danish peninsula of Jutland, constitutes an emerging risk of introduction of especially Dermacentor spp ticks and their associated pathogens from Germany and Central Europe. Here, we present the results of screening migrating birds and a golden jackal for ticks as well as ticks collected by flagging in selected urban areas in Denmark. The collected ticks were screened for exotic tick species and 38 different tick borne pathogens. We show that the risk is not just theoretical and we suggest that these introductions may have a practical public health impact.

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Source-ID: 146296245
Research output: Research - peer-review › Conference abstract for conference – Annual report year: 2018

Pathology and bacteria related to digital dermatitis in dairy cattle in all year round grazing system in Brazil
Digital dermatitis (DD) is one of the main causes of lameness in dairy cattle worldwide, and it is frequently reported in high-yielding, free stall dairy herds from regions with a temperate climate. However, DD is also observed with high prevalence in grazing cattle with a low milk yield in tropical regions. To clarify whether these differences have an impact on the etiology of the disease, we studied DD lesions from all year round grazing cattle of mixed breed in Brazil using high-throughput 16S rRNA gene sequencing and fluorescent in situ hybridization. The study included samples from 66 skin lesions and 5 healthy skins collected from five farms. Both techniques showed Treponema spp. to be the most abundant bacteria, present in all but one of the samples with minimal epidermal alterations. We identified eleven different
Treponema strains belonging to the six major phylotypes of Treponema which have all previously been identified in DD lesions. Furthermore, we identify Dichelobacter nodosus in DD lesions by gene sequencing and also by fluorescent in situ hybridization in almost half of biopsy specimens in areas with mild epithelial damage and together with Treponema. The present data support the hypothesis that Treponema constitutes the main pathogen responsible for DD, independent of the environment and region where cows are kept, and it further suggests D. nodosus as another potentially important pathogen.

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BFI (2016): BFI-level 1
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Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 3.32 SJR 1.427 SNIP 1.136
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 3.54 SJR 1.559 SNIP 1.148
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 3.94 SJR 1.772 SNIP 1.153
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
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ISI indexed (2012): ISI indexed yes
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BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 4.58 SJR 2.425 SNIP 1.233
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ISI indexed (2011): ISI indexed no
Web of Science (2011): Indexed yes
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Scopus rating (2010): SJR 2.705 SNIP 1.178
Web of Science (2010): Impact factor 4.411
Web of Science (2010): Indexed yes
Predicting and mapping human risk of exposure to Ixodes ricinus nymphs in northern Europe using climatic and environmental data

In recent years, focus on tick-borne diseases has increased as diseases such as Lyme disease and tick-borne encephalitis have become more common and represent a health problem in many parts of Scandinavia. More effective prevention of infections requires a better understanding of the factors affecting the vector abundance as well as human exposure to the vectors. Hence, there is a great need for analyses and models that can predict how vectors and their associated diseases are distributed now and possibly in the future.

As a part of the ScandTick Innovation project, we surveyed tick nymphs at 159 sites (forests and meadows) in Denmark, southern Norway and south-eastern Sweden. At each site we measured presence/absence, and used the data obtained along with environmental data from satellite images to run Boosted Regression Tree machine learning algorithms to predict overall distribution in southern Scandinavia. Together with the predicted distribution maps, we used human density maps to identify and plot areas with high risk of exposure to ticks.

The predicted distribution and the spatial variation found corresponded well with known distributions of ticks in Scandinavia (sensitivity: 91%, specificity: 60%), and we found that the model was predominantly temperature-driven. Because presence was strongly correlated with forested habitats the risk areas were much larger in Sweden and Norway compared to Denmark. When combining these distribution maps with human population density maps, we were able to quantify the proportion of people living in areas with tick presence in Scandinavia. We found that although tick nymphs were restricted to a small proportion of the modelled area, high proportions of the human populations (67-79%) lived within these same areas. The model suggests that a potential future range expansion of I. ricinus in Scandinavia is likely but may only affect a relatively small additional proportion of the human population.
tick-borne viruses (TBVs) existing worldwide. The aim of this study was to improve the epidemiological survey tools of TBVs by the development of an efficient high-throughput test to screen a wide range of viruses in ticks. In this study, we developed a new high-throughput virus-detection assay based on parallel real-time PCRs on a microfluidic system, and used it to perform a large scale epidemiological survey screening for the presence of 21 TBVs in 18,135 nymphs of *I. ricinus* collected from five European countries. This extensive investigation has (i) evaluated the prevalence of four viruses present in the collected ticks, (ii) allowed the identification of viruses in regions where they were previously undetected. In conclusion, we have demonstrated the capabilities of this new screening method that allows the detection of numerous TBVs in a large number of ticks. This tool represents a powerful and rapid system for TBVs surveillance in Europe and could be easily customized to assess viral emergence.

**General information**

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- Web of Science (2016): Impact factor 2.335
- Scopus rating (2015): CiteScore 2.12
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- Scopus rating (2014): CiteScore 2.32
- Web of Science (2014): Impact factor 2.403
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- Web of Science (2012): Impact factor 2.684
- Web of Science (2012): Indexed yes
- Web of Science (2011): Impact factor 2.441
- Web of Science (2011): Indexed yes
- Web of Science (2010): Impact factor 2.494
- Web of Science (2010): Indexed yes
- Web of Science (2009): Indexed yes
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Relative abundance and geographical variation of Culex pipiens and Culex torrentium (Diptera: Culicidae) in CO₂-baited traps in Denmark

European Culex pipiens and Culex torrentium are morphologically similar mosquito species with potentially different vector competences for pathogenic viruses. The relative abundance of the two species is therefore important for quantifying the potential for disease transmission in Denmark. Mosquitoes were sampled from 74 different sites in Denmark with CO₂ and octenol-baited suction traps. A total of 285 Culex specimens were identified to species using a restriction enzyme assay. Culex pipiens was the dominating species with 220 (77%) specimens caught at 22 different sites, while 65 (23%) specimens were identified as C. torrentium and only caught at 4 sites. The ratio of the two species differed significantly between sites with C. torrentium dominating in just a single location. Both mosquito species were predominantly caught late in the Danish mosquito season, from mid-August and onwards.

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Web of Science (2018): Indexed yes
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Scopus rating (2017): CiteScore 0.39 SJR 0.184 SNIP 0.335
Web of Science (2017): Impact factor 0.256
Web of Science (2017): Indexed yes
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Scopus rating (2016): CiteScore 0.31 SJR 0.181 SNIP 0.305
Web of Science (2016): Impact factor 0.3
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Scopus rating (2015): CiteScore 0.31 SJR 0.201 SNIP 0.299
Web of Science (2015): Impact factor 0.353
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 0.41 SJR 0.303 SNIP 0.514
Web of Science (2014): Impact factor 0.377
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 0.46 SJR 0.245 SNIP 0.658
Web of Science (2013): Impact factor 0.441
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 0.34 SJR 0.275 SNIP 0.662
Web of Science (2012): Impact factor 0.41
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 0.36 SJR 0.29 SNIP 0.586
Web of Science (2011): Impact factor 0.333
ISI indexed (2011): ISI indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 0.288 SNIP 0.507
Tag ikke engflået med hjem fra ferien

Engflåten Dermacentor reticulatus spredes hastigt i Vesteuropa, og sammen med flåten udbredes også blodparasitten Babesia canis. B. canis kan forårsage en alvorlig infektion i hunde, men parasitten kan ikke spredes af vores almindelige skovflåt. Heldigvis er engflåten endnu ikke etableret i Danmark, og man kan derfor stadigvæk trygt færdes med sin hund i den danske natur

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Contributors: Bødker, R., Kjær, L. J., Schou, K. K.
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Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
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BFI (2013): BFI-level 1
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BFI (2012): BFI-level 1
ISI indexed (2012): ISI indexed no
BFI (2011): BFI-level 1
ISI indexed (2011): ISI indexed no
BFI (2010): BFI-level 1
BFI (2009): BFI-level 1
BFI (2008): BFI-level 1
Original language: English
Source: PublicationPreSubmission
Colonization of the bovine uterus by Candida kefyr

Background. While fungal infections of the bovine uterus are well-known diseases in pregnant cattle, very limited knowledge exists on the presence and significance of fungi in the uterus of non-pregnant cows. Presence of fungi in the uterine lumen of postpartum (pp) cows has been reported, but little attention has been paid to this as most studies of the bovine pp uterus have focused on bacteria. Case presentation. Microscopy of uterine lavage cytology slides of three cows from one herd revealed the presence of numerous yeast-like organisms, which were located either free in the fluid or within macrophages. Two of the cows were around 30 days pp, while the third was 7 months pp. None of the cows had been treated with antibiotics. Culturing of the flush samples was unsuccessful, but Sanger sequencing of DNA extracted from an endometrial biopsy of one of the cows revealed the presence of Candida kefyr (Kluyveromyces marxianus). Fluorescence in situ hybridization examination of endometrial tissue sections of two cows using probes targeting 18S rRNA of the K. marxianus group was performed and revealed the presence of yeast cells on the endometrium. Histology was performed and demonstrated hyphal and non-hyphal yeast-like organisms on the surface of endometrium and in the crypts. Tissue invasion was restricted to the superficial part of the epithelium and although endometrial inflammation was present, this was mild and considered as not being caused by the fungi. One of the cows became pregnant and delivered a normal calf at term, while the two others were not bred. Conclusions. Candida kefyr is commonly isolated from milk of cows with mastitis, but has not been reported in association with other diseases of cattle. The infection was present as a monoculture in all three cows, but the fungi had only colonized the uterine lumen and the endometrial surface. Only a mild non-suppurative endometrial inflammation was present, but within the uterine luminal content, many macrophages having phagocytized yeast cells were present. Re-examination of the cows did not reveal a persistent infection, so the infection probably resolved spontaneously.
Identification of Dermacentor reticulatus Ticks Carrying Rickettsia raoultii on Migrating Jackal, Denmark

From a migrating golden jackal (Canis aureus), we retrieved 21 live male Dermacentor reticulatus ticks, a species not previously reported from wildlife in Denmark. We identified Rickettsia raoultii from 18 (86%) of the ticks. This bacterium is associated with scalp eschar and neck lymphadenopathy after tick bite syndrome among humans.
Microbiota analysis of environmental slurry and its potential role as a reservoir of bovine digital dermatitis pathogens

At present, very little information exists regarding what role the environmental slurry may play as an infection reservoir and/or route of transmission for bovine digital dermatitis (DD), a disease which is a global problem in dairy herds. To investigate, if DD-related bacteria belong to the indigenous microbiota of the dairy herd environment, we used deep amplicon sequencing of the 16S rRNA gene in 135 slurry samples collected from different sites in 22 dairy farms, with and without DD-infected cows. Both the general bacterial populations as well as digital dermatitis-associated Treponema were targeted in this study. The results revealed significant differences in the bacterial communities between the herds, with only 12 bacterial taxa shared across at least 80% of all the individual samples. These differences in the herd microbiota appeared to reflect mainly between-herd variation. Not surprisingly, the slurry was dominated by ubiquitous gastrointestinal bacteria, such as Ruminococcaceae and Lachnospiraceae. Despite the low relative abundance of spirochetes, which ranged from 0 to 0.6%, we were able to detect small amounts of bacterial DNA from DD-associated treponemes in the slurry. However, the DD-associated Treponema spp. were only detected in samples from herds with reported problems of DD. These data indicate that treponemes involved in the pathogenesis of DD are not part of the normal environmental microflora in dairy herds without clinical DD and, consequently, that slurry is not a primary reservoir of infection.

Importance Bovine digital dermatitis (DD), a dermal disease which causes lameness in dairy cattle, is a serious problem worldwide. To control this disease, the infection reservoirs and transmission routes of DD pathogens need to be clarified. The dairy herd slurry may be a possible pathogen reservoir of DD-associated bacteria. The rationale for the present study was, therefore, to examine whether DD-associated bacteria are always present in slurry or if they are only found in DD-affected herds. The results strongly indicated that DD Treponema are not part of the indigenous slurry and, therefore, do not comprise an infection reservoir in healthy herds. This study applied next-generation sequencing technology to decipher the microbial compositions of environmental slurry of dairy herds with and without digital dermatitis.
Modelling risk of tick exposure in southern Scandinavia using machine learning techniques, satellite imagery, and human population density maps

Vector-borne diseases such as Lyme disease and tick-borne encephalitis have become more common in recent decades and present a real health problem in many parts of Europe. Risk assessment, control, and prevention of these diseases require a better understanding of vector abundance as well as risk factors determining human exposure to ticks. There is a great need for analyses and models that can predict how vectors and their associated diseases are distributed and how this relates to high risk areas for human exposure. As a part of the ScandTick Innovation project, we surveyed ticks at approximately 30 sites (forests and meadows) in each of Denmark, southern Norway and south-eastern Sweden. At each site we measured presence/absence of ticks, and used the data obtained along with environmental satellite images to run Boosted Regression Tree machine learning algorithms to predict overall spatial distribution (probability of presence) in southern Scandinavia. Together with the predicted distribution maps, we used human density maps to determine areas with high risk of exposure to ticks. For nymphs, the predicted distribution found corresponded well with known distributions of ticks in Scandinavia, with more widespread distribution in Denmark compared to Norway and Sweden. In the Norwegian region, probability of presence was markedly higher nearer the coastline and the data shows a latitudinal boundary in the Swedish region above which probability of presence was low or close to zero. Presence of larvae was much more clustered in the observed data, which was also reflected in the predicted distribution maps for the region. Whereas the predicted distribution of larvae was mostly even throughout Denmark, larvae were primarily around the coastlines in Norway and Sweden. When combining these distribution maps with human population density maps and accounting for area accessibility, we could assess the proportion of the population living in areas where ticks were present. Our data showed that although ticks are found in a limited proportion of the total region area (particularly for Norway and Sweden), areas with high population densities tend to overlap with these zones. Machine learning techniques allow us to predict for larger areas without having to perform extensive sampling all over the region in question, and we were able to produce models and maps with high predictive value. The results from these models help us pinpoint areas with high risk of exposure to ticks and thus potentially tick-borne diseases.

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Modelling tick abundance using machine learning techniques and satellite imagery

Recently, focus on tick-borne diseases has increased as diseases such as Lyme disease and tickborne encephalitis have become more widespread and represent a real health problem in many parts of Europe. Effective control and prevention of these diseases requires a better understanding of the factors affecting the vectors. There is a great need for analyses and models that can predict how vectors and their associated diseases are distributed now and possibly in the future. As a part of the ScandTick Innovation project, we surveyed and collected ticks at approximately 30 sites in each of Denmark, southern Norway and south-eastern Sweden. At each site we measured presence/absence and relative tick abundance using north- and east-facing line transect, where number of larvae, nymphs and adult females and males were counted at eight 50 m transects. We used the data obtained along with environmental satellite images to run Boosted Regression Tree machine learning algorithms to predict overall distribution (presence/absence of ticks) and relative tick abundance of nymphs and larvae in southern Scandinavia. For nymphs, the predicted abundance had a positive correlation with observed abundance and the spatial variation found corresponded well with known abundance and distributions of ticks in Scandinavia, with higher abundance and more widespread distribution in Denmark compared to Norway and Sweden. Because abundance was strongly correlated with forested habitats the risk areas were much larger in Sweden and Norway compared to Denmark. In both the Norwegian and Swedish regions, abundance was markedly higher nearer the coastline. Presence of larvae was much more clustered in the observed data, which was also reflected in the predicted abundance and distribution maps for the region. Whereas the predicted distribution of larvae was mostly even throughout Denmark, it was primarily around the coastlines in Norway and Sweden. Abundance was fairly low overall except in some fragmented patches corresponding to forested habitats in the region. Machine learning techniques allow us to predict for larger areas without having to perform extensive sampling all over the region in question. The results from these models can be used in epidemiological models and can help us determine areas under risk of disease transmission and help us interpret human incidence data. Next step will be to analyze the collected ticks for pathogens and using the same machine learning techniques to develop prevalence maps of the ScandTick region.

Predicting tick abundance in Southern Scandinavia using machine learning techniques and satellite imagery – a part of the ScandTick Innovation project

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Research output: Research - peer-review » Conference abstract for conference – Annual report year: 2017

Presence and localization of bacteria in the bovine endometrium postpartum using fluorescence in situ hybridization

The aim of this study was to investigate bacterial invasiveness of the bovine endometrium during the postpartum period. Fluorescence in situ hybridization was applied to endometrial biopsies using probes for Fusobacterium necrophorum, Porphyromonas levi, Trueperella pyogenes, Escherichia coli and a probe for bacteria in general (the overall domain Bacteria) to determine their tissue localization. Holstein cows were sampled at three time points postpartum (T1: 4±12 days postpartum, T2: 24±32 days postpartum and T3: 46±54 days postpartum). At T1, cows were clinically scored as having a uterine infection based on presence of a brownish, fetid vaginal discharge or as normal if having normal lochia.

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Source-ID: 128790641
Research output: Research - peer-review » Conference abstract for conference – Annual report year: 2017
An endometrial biopsy was taken from all cows at T1 (n = 57). Endometrial biopsies were taken from the same cows at T2 and T3 if allowed by the size of the cervical canal and if the cow had not been inseminated. Fifty and 39 biopsies were obtained at T2 and T3, respectively. The biopsies were evaluated for inflammation and for presence and localization of bacteria. When analyzed by the probe for the entire domain Bacteria, bacteria were found in most biopsies irrespectively of time (T1: 79.0%, T2: 82.0%, T3: 89.7%). Fusobacterium necrophorum and Porphyromonas levii were often present in the endometrium at T1 (61.1% and 47.8%, respectively), but the prevalence decreased significantly over time. Trueperella pyogenes and Escherichia coli were less prevalent at T1 (8.8% and 10.5%, respectively) and their prevalence also decreased significantly over time. Fusobacterium necrophorum and Porphyromonas levii were often co-localized intraepithelially or in the lamina propria. Trueperella pyogenes and Escherichia coli were located only on the endometrial surface. Due to the high prevalence of tissue invasiveness, these findings emphasize the importance of Fusobacterium necrophorum and Porphyromonas levii in postpartum uterine disease of cattle and indicate that tissue invasiveness is an important aspect of the pathogenesis.
Transmission differentials for multiple pathogens as inferred from their prevalence in larva, nymph and suit of Ixodes ricinus (Acari: Ixodidae)

Ixodes ricinus serves as vector for a range of microorganisms capable of causing clinical illness in humans. The microorganisms occur in the same vector populations and are generally affected by the same tick-host interactions. Still, the instars have different host preferences which should manifest in different transmission patterns for various microorganisms in the tick populations, i.e., most microorganisms increase in prevalence rate from larvae to nymphs because their reservoirs are among small mammals and birds that serve as blood hosts for larvae. Other microorganisms, like Anaplasma phagocytophilum, mainly increase in prevalence rates from nymphs to adults, because their reservoirs are larger ungulates that serve as primary blood hosts for nymphs and adults. We sampled a representative sample of ticks from 12 locations on Zealand and Funen, Denmark, and investigated the differences in prevalence rate of infection in larvae, nymphs and adults for multiple pathogens. Prevalence of infection for larvae, nymphs and adults, respectively, was: 0, 1.5 and 4.5% for Borrelia burgdorferi; 0, 4.2 and 3.9% for Borrelia garinii; 0, 6.6 and 6.1% for Borrelia afzelii; 0, 0 and 0.6% for Borrelia valaisiana; 0, 3.7 and 0.6% for Borrelia spielmanii; 0, 0.7 and 1.2% for Babesia divergens; 0, 0, 0.6% for Babesia venatorum; 0, 1.5 and 6.1% for A. phagocytophilum. The results were in general compatible with the hypothesis i.e., that differences in blood host for larvae and nymphs define differences in transmission of infectious agents, but other factors than differences in blood hosts between larvae and nymphs may also be important to consider.

General information
State: Published
Organisations: National Veterinary Institute, Bacteriology & Parasitology, Epidemiology, University of Copenhagen, Agence nationale de la sécurité sanitaire, alimentation, environnement et travail
Contributors: Jensen, P. M., Christoffersen, C. S., Moutailler, S., Michelet, L., Schou, K. K., Bødker, R.
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Volume: 71
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Vectorborne zoonoses

General information
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Organisations: National Veterinary Institute, Epidemiology, Bacteriology & Parasitology, Technical University of Denmark
Contributors: Bødker, R., Vrbová, E., Schou, K. K.
An investigation of the microbiota in uterine flush samples and endometrial biopsies from dairy cows during the first 7 weeks postpartum

Metritis and endometritis commonly occur in dairy cows after calving. Although numerous studies have been performed to identify the causative pathogens, a complete overview has not been done. Metagenomic studies have analyzed the bacterial populations of uterine flush samples from postpartum (pp) dairy cows, but the microbiota in the uterine luminal fluid may differ from the microbiota of the endometrium itself, and important putative pathogens may have been overlooked. In the present study, we compared the microbiota of the uterine lumen and the endometrium of healthy, metritic, and endometritic cows. Samples were collected from 68 Holstein dairy cows at 1, 4, and 7 weeks pp, and the data were analyzed by deep sequencing of the V1 and V2 hypervariable regions of the 16S ribosomal RNA gene. The results showed that Porphyromonadaceae, Fusobacteriaceae, Leptotrichiaceae, and Mycoplasmataceae may be associated with uterine disease. The microbiota of the uterine flush samples and the endometrial biopsies were correlated, but the microbiota of the biopsies was more diverse. Fusobacteriaceae and Leptotrichiaceae were not observed in the biopsies at week 7, whereas they accounted for 20% and 13%, respectively, of the bacterial populations in the flush samples. The Mycoplasmataceae family was observed in much higher quantity in the flush samples than in the biopsies of the endometritis groups at weeks 4 and 7. Our findings support the observations of previous metagenomic studies and illustrate the importance of including endometrial biopsies to obtain more detailed knowledge of the pp uterine microbiota.
A novel approach to probe host-pathogen interactions of bovine digital dermatitis, a model of a complex polymicrobial infection

Polymicrobial infections represent a great challenge for the clarification of disease etiology and the development of comprehensive diagnostic or therapeutic tools, particularly for fastidious and difficult-to-cultivate bacteria. Using bovine digital dermatitis (DD) as a disease model, we introduce a novel strategy to study the pathogenesis of complex infections. The strategy combines meta-transcriptomics with high-density peptide-microarray technology to screen for in vivo-expressed microbial genes and the host antibody response at the site of infection. Bacterial expression patterns supported the assumption that treponemes were the major DD pathogens but also indicated the active involvement of other phyla (primarily Bacteroidetes). Bacterial genes involved in chemotaxis, flagellar synthesis and protection against oxidative and acidic stress were among the major factors defining the disease. The extraordinary diversity observed in bacterial
expression, antigens and host antibody responses between individual cows pointed toward microbial variability as a hallmark of DD. Persistence of infection and DD reinfection in the same individual is common; thus, high microbial diversity may undermine the host's capacity to mount an efficient immune response and maintain immunological memory towards DD. The common antigenic markers identified here using a high-density peptide microarray address this issue and may be useful for future preventive measures against DD.

General information
State: Published
Organisations: Department of Systems Biology, Center for Biological Sequence Analysis, National Veterinary Institute, Section for Bacteriology, Pathology and Parasitology, Metagenomics, Hospital of Southern Jutland, Schafer-N
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  Scopus rating (2017): CiteScore 4.08 SJR 2.11 SNIP 1.151
  Web of Science (2017): Impact factor 3.73
  Web of Science (2017): Indexed yes
  BFI (2016): BFI-level 1
  Scopus rating (2016): CiteScore 4.05 SJR 2.163 SNIP 1.096
  Web of Science (2016): Impact factor 3.729
  Web of Science (2016): Indexed yes
  BFI (2015): BFI-level 1
  Scopus rating (2015): CiteScore 4.3 SJR 2.348 SNIP 1.159
  Web of Science (2015): Impact factor 3.867
  Web of Science (2015): Indexed yes
  BFI (2014): BFI-level 1
  Scopus rating (2014): CiteScore 4.18 SJR 2.327 SNIP 1.199
  Web of Science (2014): Impact factor 3.986
  Web of Science (2014): Indexed yes
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  ISI indexed (2013): ISI indexed yes
  Web of Science (2013): Indexed yes
  BFI (2012): BFI-level 1
  Scopus rating (2012): CiteScore 4.61 SJR 2.236 SNIP 1.243
  Web of Science (2012): Impact factor 4.397
  ISI indexed (2012): ISI indexed yes
  Web of Science (2012): Indexed yes
  BFI (2011): BFI-level 1
  Scopus rating (2011): CiteScore 4.38 SJR 2.307 SNIP 1.191
  Web of Science (2011): Impact factor 4.073
  ISI indexed (2011): ISI indexed yes
  Web of Science (2011): Indexed yes
Communicating spatial risk of tick-borne infections - Creating a ScandTick Innovation website based on surveillance data

General information
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Organisations: National Veterinary Institute, Section for Epidemiology, Section for Bacteriology, Pathology and Parasitology
Contributors: Clausen, C. G., Schou, K. K., Kirkeby, C., Bødker, R.
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Source: PublicationPreSubmission
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Research output: Research - peer-review › Poster – Annual report year: 2016

Communicating spatial risk of tick-borne infections: Creating a ScandTick Innovation website based on surveillance data

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Contributors: Clausen, C. G., Schou, K. K., Kirkeby, C., Bødker, R.
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Communicating spatial variation in tick-borne pathogen prevalence through a website based on national surveillance data

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Contributors: Clausen, C. G., Schou, K. K., Kirkeby, C. T., Bødker, R.
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Identification of common bacterial antigenic markers from bovine digital dermatitis lesions using meta-transcriptomics in combination with high-density peptide-microarrays

Bovine digital dermatitis (DD) is the most important infectious cause of lameness in dairy cattle, and a major contributing factor to welfare problems and economic losses in the dairy cattle industry worldwide. DD is a disease that involves chronic dermal inflammatory processes and destruction of collagenous and connective tissues. Multiple Treponema species, many of which are not-yet-cultivable, are strongly implicated in disease progression. Despite the economic and welfare importance of this disease, no effective vaccine is available; and there is presently very little knowledge concerning efficacious immunoprophylactic antigens against DD.

It is highly likely that DD-associated treponemes possess considerable antigenic variation, as cows exhibit a variable humoral response against different isolates of Treponema. Hence, combinations of antigens from multiple Treponema species should be used for the development of disease prevention measures. As treponemes from DD lesions are extremely difficult to culture, identification of these antigens is challenging. To circumvent this problem, we studied the in situ gene expression patterns of the microbiome in DD-affected skin lesions and the host antibody response directed at the site of infection. By metatranscriptomics we measured the in situ genome-wide transcriptome of the bacterial population in DD-affected skin lesions from 21 dairy cows. From the transcriptome data, we identified a panel of Treponema genes that were highly expressed in multiple animals, and we monitored the host immune response to these target genes using high-density peptide microarrays. By this approach, we identified a small group of antigenic proteins, which were expressed in the majority of the samples, and demonstrated antigenicity when screened against sera from infected animal. Future studies will show if these proteins represent candidates for the development of novel biomarkers or vaccines.

General information
State: Published
Organisations: National Veterinary Institute, Section for Bacteriology, Pathology and Parasitology, Center for Biological Sequence Analysis, Department of Bio and Health Informatics, Immunoinformatics and Machine Learning, Metagenomics, Schafer-N, Technical University of Denmark
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Research output: Research - peer-review › Conference abstract for conference – Annual report year: 2016

Potential bacterial core species associated with digital dermatitis in cattle herds identified by molecular profiling of interdigital skin samples

General information
State: Published
Organisations: National Veterinary Institute, Section for Bacteriology, Pathology and Parasitology, Center for Biological Sequence Analysis, Department of Bio and Health Informatics, Immunoinformatics and Machine Learning, Metagenomics, Schafer-N, Technical University of Denmark
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Peer-reviewed: Yes
Research output: Research - peer-review › Conference abstract for conference – Annual report year: 2016
Although treponemes are consistently identified in tissue from bovine digital dermatitis (DD) lesions, the definitive etiology of this debilitating polymicrobial disease is still unresolved. To study the microbiomes of 27 DD-infected and 10 healthy interdigital skin samples, we used a combination of different molecular methods. Deep sequencing of the 16S rRNA gene variable regions V1–V2 showed that Treponema, Mycoplasma, Fusobacterium and Porphyromonas were the genera best differentiating the DD samples from the controls. Additional deep sequencing analysis of the most abundant genus, Treponema, targeting another variable region of the 16S rRNA gene, V3–V4, identified 15 different phylotypes, among which Treponema phagedenis-like and Treponema refringens-like species were the most abundant. Although the presence of Treponema spp., Fusobacterium necrophorum and Porphyromonas levii was confirmed by fluorescence in situ hybridization (FISH), the results for Mycoplasma spp. were inconclusive. Extensive treponemal epidermal infiltration, constituting more than 90% of the total bacterial population, was observed in 24 of the 27 DD samples. F. necrophorum and P. levii were superficially located in the epidermal lesions and were present in only a subset of samples. RT-qPCR analysis showed that treponemes were also actively expressing a panel of virulence factors at the site of infection. Our results further support the hypothesis that species belonging to the genus Treponema are major pathogens of DD and also provide sufficient clues to motivate additional research into the role of M. fermentans, F. necrophorum and P. levii in the etiology of DD.
Prediciting spatial distribution of pathogenstransmitted by ticks in northern Europe

General information
State: Published
Organisations: National Veterinary Institute, Section for Epidemiology, Section for Bacteriology, Pathology and Parasitology, Wageningen University & Research, National Veterinary Institute, ANSES - French Agency for Food, Environmental and Occupational Health & Safety, Central Veterinary Institute, Animal and Plant Health Agency
Contributors: Cuellar, A. C., Schou, K. K., Moutailler, S., Fach, P., Delannoy, S., van der Wal, F., de Koeier, A., Chirico, J., Aspan, A., Juremalm, M., Mansfield, K., Phipps, P., Fooks, T., Badker, R.
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Title of host publication: 3rd Conference on Neglected Vectors and Vector-Borne Diseases (EurNegVec): with MC and WG Meeting of the COST Action TD1303 : Abstract book
Predicting spatial distribution of pathogens transmitted by ticks in Northern Europe

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State: Published
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Event information
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Predicting spatial prevalence of tick pathogens in Northern Europe using satellite imagery

General information
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Organisations: National Veterinary Institute, Section for Epidemiology, Section for Bacteriology, Pathology and Parasitology, ANSES - French Agency for Food, Environmental and Occupational Health & Safety, Wageningen University & Research, Animal and Plant Health Agency, National Veterinary Institute
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Spatial risk of tick borne infections – creating a ScandTick Innovation website for both the public and the health sector based on surveillance data

General information
State: Published
Organisations: National Veterinary Institute, Section for Epidemiology, Section for Bacteriology, Pathology and Parasitology
Contributors: Clausen, C. G., Schou, K. K., Kirkeby, C., Bødker, R.
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**Vectorborne zoonoses**

**General information**
State: Published
Organisations: National Veterinary Institute, Section for Epidemiology, Section for Bacteriology, Pathology and Parasitology
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Place of publication: Søborg
Publisher: National Food Institute, Technical University of Denmark (Annual Report on Zoonoses in Denmark).
Electronic versions:
Rapport_Annual_Report_On_Zoonoses_in_Denmark_2015_FINAL.pdf
Research output: Commissioned › Report chapter – Annual report year: 2016

**112 Presence of bacteria in the endometrium and oviduct of cows with pyometra as detected by fluorescence in situ hybridization**

The objective of the study was to identify the location of the present bacteria in the uterus and oviducts of cows with pyometra. Pyometra is one of the postpartum infectious diseases in cattle that can result in infertility and thereby affect reproduction performance. Reproductive tracts (n = 21) were collected at a slaughterhouse in Denmark and sent to The University of Copenhagen for examination and sampling. The uteri were included in the study when the following criteria were met: the cow was more than 21 days postpartum, the uterus was distended with pus, the cervix was closed, and a corpus luteum was present in one or both ovaries. A full thickness uterine tissue sample from the previous pregnant horn and both oviducts were sampled and then fixed in formalin. The tissues were trimmed, processed by routine methods, embedded in paraffin, sectioned at 3 microns, and prepared for fluorescence in situ hybridization using a probe targeting the 16S ribosomal RNA of the domain bacteria (i.e. targeting all bacteria regardless of species). Using fluorescence microscopy, the presence of bacteria within or on the surface of the endometrium and in the oviducts were noted. The endometrial biopsies from all cows (n = 21) contained bacteria, while 75% (16/21) of the cows had bacteria in one or both oviducts. The bacteria were located on the luminal surface and in the lamina propria in 38.1% (8/21) of the uterine biopsies. In the remaining 62% of the uterine biopsies, the bacteria were only located above the basal membrane. Regarding the oviduct biopsies, the bacteria were located on the luminal surface and in lamina propria in 9.5% (2/21) of the biopsies, whereas the bacteria were located only above the basal membrane in 90.5% of the biopsies. In conclusion, 1) bacteria are present in the uteri and oviducts of cows with pyometra and 2) the bacteria are primarily located on the luminal epithelia surface above the basal membrane. Further analyses will investigate which specific species of bacteria that are located in the lamina propria of the uterine and oviduct biopsies.

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Organisations: National Veterinary Institute, Section for Bacteriology, Pathology and Parasitology, University of Copenhagen, Technical University of Denmark
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Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 1.75 SJR 0.681 SNIP 0.766
Concurrent host-pathogen gene expression in the lungs of pigs challenged with Actinobacillus pleuropneumoniae

Background: Actinobacillus pleuropneumoniae causes pleuropneumonia in pigs, a disease which is associated with high morbidity and mortality, as well as impaired animal welfare. To obtain in-depth understanding of this infection, the interplay between virulence factors of the pathogen and defense mechanisms of the porcine host needs to be elucidated. However, research has traditionally focused on either bacteriology or immunology; an unbiased picture of the transcriptional responses can be obtained by investigating both organisms in the same biological sample. Results: Host and pathogen responses in pigs experimentally infected with A. pleuropneumoniae were analyzed by high-throughput RT-qPCR. This approach allowed concurrent analysis of selected genes encoding proteins known or hypothesized to be important in the acute phase of this infection. The expression of 17 bacterial and 31 porcine genes was quantified in lung samples obtained within the first 48 hours of infection. This provided novel insight into the early time course of bacterial genes involved in synthesis of pathogen-associated molecular patterns (lipopolysaccharide, peptidoglycan, lipoprotein) and genes involved in pattern recognition (TLR4, CD14, MD2, LBP, MYD88) in response to A. pleuropneumoniae. Significant up-regulation of proinflammatory cytokines such as IL1B, IL6, and IL8 was observed, correlating with protein levels,
infection status and histopathological findings. Host genes encoding proteins involved in iron metabolism, as well as bacterial genes encoding exotoxins, proteins involved in adhesion, and iron acquisition were found to be differentially expressed according to disease progression. By applying laser capture microdissection, porcine expression of selected genes could be confirmed in the immediate surroundings of the invading pathogen. Conclusions: Microbial pathogenesis is the product of interactions between host and pathogen. Our results demonstrate the applicability of high-throughput RT-qPCR for the elucidation of dual-organism gene expression analysis during infection. We showed differential expression of 12 bacterial and 24 porcine genes during infection and significant correlation of porcine and bacterial gene expression. This is the first study investigating the concurrent transcriptional response of both bacteria and host at the site of infection during porcine respiratory infection.

General information
State: Published
Organisations: National Veterinary Institute, Section for Immunology and Vaccinology, Section for Bacteriology, Pathology and Parasitology
Contributors: Brogaard, L., Schou, K. K., Heegaard, P. M. H., Hansen, M. S., Jensen, T. K., Skovgaard, K.
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BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 4.08 SJR 2.11 SNIP 1.151
Web of Science (2017): Impact factor 3.73
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BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 4.05 SJR 2.163 SNIP 1.096
Web of Science (2016): Impact factor 3.729
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BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 4.3 SJR 2.348 SNIP 1.159
Web of Science (2015): Impact factor 3.867
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 4.18 SJR 2.327 SNIP 1.199
Web of Science (2014): Impact factor 3.986
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BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 4.39 SJR 2.195 SNIP 1.188
Web of Science (2013): Impact factor 4.041
ISI indexed (2013): ISI indexed yes
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Web of Science (2012): Impact factor 4.397
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 4.38 SJR 2.307 SNIP 1.191
Web of Science (2011): Impact factor 4.073
High-throughput Gene Expression Analysis In Pigs As Model For Respiratory Infections

Influenza A virus infections have great impact on human health and welfare and significant resources are linked to influenza epidemics due to excess hospitalizations and lost productivity. Up to 15% of the human population is affected when Influenza spreads around the world in seasonal epidemics (WHO).

Animal models are essential in understanding the mechanisms involved in human infectious disease and for the development of effective prevention and treatment strategies. It is increasingly realized that large animal models like the pig are exceptionally human like and serve as an excellent model for disease and inflammation. Pigs are fully susceptible to human influenza, and have been demonstrated to be involved in influenza evolution and ecology. Pigs share many similarities with humans regarding lung physiology and innate immune cell infiltration of the respiratory system and thus seem to be an obvious large animal model for respiratory infections. This study aimed at providing a better understanding of the involvement of circulating non-coding RNA and innate immune factors in porcine blood leukocytes during influenza virus infection. By employing the pig as a model we were able to perform highly controlled experimental infections and to study changes of symptoms, viral titer, and expression of microRNAs/mRNAs as the influenza infection progresses in time, generating information that would be difficult to obtain from human patients.

The Gram-negative bacterium Actinobacillus pleuropneumoniae causes pneumonia in pigs, a disease which is associated with high morbidity and mortality, as well as impaired animal welfare. The rapidly evolving pneumonia is characterized by large areas of lung necrosis resulting from the combined effect of tissue damage caused by the bacteria, and a strong proinflammatory immune response. To obtain in-depth understanding of this infection, concurrent gene expression of host and pathogen in lung samples collected from pigs experimentally infected with A. pleuropneumoniae was studied. We applied high-throughput RT-qPCR for the simultaneous analysis of host and pathogen gene expression. This parallel analysis was done in lung tissue samples as well as in the immediate surroundings of infection loci after laser capture microdissection. Regulation of gene expression of several immune factors was observed in agreement with protein levels of these factors in lung tissue, infection status and histopathological findings.

Preliminary data on the presence of bacteria in the uterus of pregnant cows

Bacterial invasion of the uterus during the postpartum period has been well described. Recent papers using 16S rRNA gene sequencing techniques suggest that the nonpregnant uterus contains a diverse flora of bacteria that are not necessarily pathogenic. In contrast, the pregnant uterus has until now been considered a sterile environment. The aim of the present study was to investigate whether bacteria were present in the uteri of pregnant cows. Uteri from pregnant, slaughtered animals (n = 47) were sampled. The surface of the uterus was wiped with alcohol, flame sterilized, and cut open with sterile scissors. Samples were taken from the endometrium and from the placentomes. The samples were embedded in paraffin, sectioned at 3 microns, and prepared for fluorescence in situ hybridization using a probe targeting the 16S rRNA of the domain bacteria, so that all bacteria regardless of species were visualised. Using fluorescence microscopy, the presence of bacteria within or on the surface of the endometrium and within the placentomes was noted. The stage of pregnancy was estimated to range from 26 to 263 days by measuring the size of the embryo or fetus. The endometrial samples from 85.1% (40/47) of pregnant cows contained bacteria. In 22 cows, the bacteria were localised within the endometrial tissue, whereas in the remaining 18 cows, the bacteria were on the epithelial surface. Placental samples were obtained from 43 cows, and 76.7% (33/43) of these contained bacteria. The presence of bacteria in the pregnant uterus may suggest that a cow can carry a pregnancy despite the presence of few potentially pathogenic bacteria or that normal flora exist in the uterus as in, for example, the vagina. In conclusion, bacteria were present in the endometrium and placenomes of pregnant cows. Further analyses using rRNA gene sequencing techniques will aim to
confirm the presence of bacteria in the bovine pregnant uterus and to investigate which species of bacteria are present in the uterus during pregnancy.

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Scopus rating (2017): CiteScore 1.75 SJR 0.681 SNIP 0.766
Web of Science (2017): Impact factor 2.105
Web of Science (2017): Indexed yes
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Scopus rating (2016): CiteScore 1.88 SJR 0.788 SNIP 0.895
Web of Science (2016): Impact factor 2.656
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 1.9 SJR 0.852 SNIP 0.96
Web of Science (2015): Impact factor 2.135
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 2.45 SJR 1.054 SNIP 1.101
Web of Science (2014): Impact factor 2.4
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 2.04 SJR 0.917 SNIP 0.961
Web of Science (2013): Impact factor 2.577
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 2.27 SJR 0.916 SNIP 1.032
Web of Science (2012): Impact factor 2.583
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 1.9 SJR 0.845 SNIP 0.833
Web of Science (2011): Impact factor 2.109
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 0.872 SNIP 0.925
Web of Science (2010): Impact factor 2.553
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.896 SNIP 1.024
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 0.796 SNIP 0.794
Scopus rating (2007): SJR 0.883 SNIP 0.924
Scopus rating (2006): SJR 0.676 SNIP 0.839
Scopus rating (2005): SJR 0.499 SNIP 0.576
Scopus rating (2004): SJR 0.483 SNIP 0.638
Scopus rating (2003): SJR 0.454 SNIP 0.557
Revisiting bovine pyometra—New insights into the disease using a culture-independent deep sequencing approach

The bacteria present in the uterus during pyometra have previously been studied using bacteriological culturing. These studies identified Fusobacterium necrophorum and Trueperella pyogenes as the major contributors to the pathogenesis of pyometra. However, an increasing number of culture-independent studies have demonstrated that the bacterial diversity in most environments is underestimated in culture-based studies. Consequently, fastidious pyometra-associated pathogens may have been overlooked. Therefore, the primary purpose of this study was to investigate the diversity of bacteria in the uterus of cows with pyometra by using culture-independent 16S rRNA PCR combined with next generation sequencing. We investigated the microbial composition in the uterus of 21 cows with pyometra, which were obtained from a Danish slaughterhouse. Similar to the observations from the culture studies, Fusobacteriaceae, the family that F. necrophorum belongs to, was the operational taxonomic unit (OTU) observed in the largest quantities. By contrast, the Actinomycetaceae family, which includes T. pyogenes, constituted only 1% of the total number of reads. Thus we cannot confirm the previously reported role of species from this family in the pathogenesis of pyometra. Finally, we identified a large number of sequences representing three families of Gram-negative bacteria in the pyometra samples: Porphyromonadaceae, Mycoplasmataceae, and Pasteurellaceae. It is likely that these families comprise potential pathogenic species of a fastidious nature, which have been overlooked in previous studies. Our results increase the knowledge of the complexity of the pyometra microbiota and suggest that pathogens in addition to F. necrophorum may be involved in the pathogenesis of pyometra. (C) 2014 Elsevier B.V. All rights reserved.

General information

State: Published
Organisations: National Veterinary Institute, Section for Bacteriology, Pathology and Parasitology, University of Copenhagen
Number of pages: 6
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Publication information

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- BFI (2019): BFI-level 2
- Web of Science (2019): Indexed yes
- BFI (2018): BFI-level 2
- Web of Science (2018): Indexed yes
- BFI (2017): BFI-level 2
- Scopus rating (2017): CiteScore 2.7 SJR 1.175 SNIP 1.241
- Web of Science (2017): Impact factor 2.524
- Web of Science (2017): Indexed yes
- BFI (2016): BFI-level 2
- Scopus rating (2016): CiteScore 2.65 SJR 1.363 SNIP 1.206
- Web of Science (2016): Impact factor 2.628
- Web of Science (2016): Indexed yes
- BFI (2015): BFI-level 2
- Scopus rating (2015): CiteScore 2.56 SJR 1.413 SNIP 1.21
- Web of Science (2015): Impact factor 2.564
Detection of polytreponemal infection in three cases of porcine ulcerative stomatitis by Fluorescent in situ hybridization

General information
State: Published
Organisations: National Veterinary Institute, Section for Bacteriology, Pathology and Parasitology, Utrecht University, University of Veterinary Medicine Hannover, Foundation
Contributors: Jensen, T. K., Strijkstra, G., Gruys, E., Baumgärtner, W., Schou, K. K., Boye, M.
Publication date: 2014
Peer-reviewed: Yes
Event: Abstract from Cutting Edge Pathology, Berlin, Germany.
Electronic versions:
ECVP_and_ESVP_Abstract_Treponema_in_pigs_DTU_Vet.pdf
Source: PublicationPreSubmission
Source-ID: 99797255
Research output: Research - peer-review › Conference abstract for conference – Annual report year: 2014

Discovery of Bovine Digital Dermatitis-Associated Treponema spp. in the Dairy Herd Environment by a Targeted Deep-Sequencing Approach.
The bacteria associated with the infectious claw disease bovine digital dermatitis (DD) are spirochetes of the genus Treponema; however, their environmental reservoir remains unknown. To our knowledge, the current study is the first report of the discovery and phylogenetic characterization of rRNA gene sequences from DD-associated treponemes in the dairy herd environment. Although the spread of DD appears to be facilitated by wet floors covered with slurry, no DD-associated treponemes have been isolated from this environment previously. Consequently, there is a lack of knowledge about the spread of this disease among cows within a herd as well as between herds. To address the issue of DD infection reservoirs, we searched for evidence of DD-associated treponemes in fresh feces, in slurry, and in hoof lesions by deep sequencing of the V3 and V4 hypervariable regions of the 16S rRNA gene coupled with identification at the operational-taxonomic-unit level. Using treponeme-specific primers in this high-throughput approach, we identified small amounts of DNA (on average 0.6% of the total amount of sequence reads) from DD-associated treponemes in 43 of 64 samples from slurry and cow feces collected from six geographically dispersed dairy herds. Species belonging to the Treponema denticola/Treponema pedis-like and Treponema phagedenis-like phylogenetic clusters were among the most prevalent treponemes in both the dairy herd environment and the DD lesions. By the high-throughput approach presented here, we have demonstrated that cow feces and environmental slurry are possible reservoirs of DD-associated treponemes. This method should enable further clarification of the etiopathogenesis of DD.

General information
State: Published
Organisations: National Veterinary Institute, Section for Bacteriology, Pathology and Parasitology
Contributors: Schou, K. K., Weiss Nielsen, M., Ingerslev, H., Boye, M., Jensen, T. K.
Number of pages: 6
Pages: 4427-4432
Publication date: 2014
Peer-reviewed: Yes

Publication information
Journal: Applied and Environmental Microbiology
Volume: 80
Issue number: 14
ISSN (Print): 0099-2240
Ratings:
BFI (2019): BFI-level 2
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 3.99
Web of Science (2017): Impact factor 3.633
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.08
Web of Science (2016): Impact factor 3.807
Genetic diversity of Treponemes in dairy herds and their surrounding environment

General information
State: Published
Organisations: National Veterinary Institute, Section for Bacteriology, Pathology and Parasitology
Contributors: Weiss Nielsen, M., Jensen, T. K., Ingerslev, H., Schou, K. K.
Number of pages: 1
Publication date: 2014
Peer-reviewed: Yes
Event: Poster session presented at Gordon Research Conference on Spirochetes, Ventura, CA, United States.
Electronic versions:
Poster_Ventura_Biology_of_Spirochetes_2014.pdf

High-throughput gene expression analysis in pigs as model for respiratory infections
Animal models are essential in understanding the mechanisms involved in human infectious disease and for the development of effective prevention and treatment strategies. It is increasingly realized that large animal models like the pig are exceptionally human like and serve as an excellent model for disease and inflammation. Pigs are fully susceptible to human influenza, and have been demonstrated to be involved in influenza evolution and ecology. Pigs share many similarities with humans regarding lung physiology and innate immune cell infiltration of the respiratory system and thus seem to be an obvious large animal model for respiratory infections. This study aimed at providing a better understanding of the involvement of circulating non-coding RNA and innate immune factors in porcine blood leukocytes during influenza virus infection. By employing the pig as a model we were able to perform highly controlled experimental infections and to study changes of symptoms, viral titer, and expression of microRNAs/mRNAs as the influenza infection progresses in time, generating information that would be difficult to obtain from human patients.

General information
State: Published
Organisations: National Veterinary Institute, Section for Immunology and Vaccinology, Section for Bacteriology, Pathology and Parasitology, Section for Virology
Contributors: Skovgaard, K., Brogaard, L., Schou, K. K., Larsen, L. E., Mortensen, S., Dürrwald, R., Schengel, M., Heegaard, P. M. H.
Publication date: 2014
Peer-reviewed: Yes
Event: Abstract from Conferences and Workshops of COST Action BM1308, Munich, Germany.
Electronic versions:
High_throughput_gene_expression_analysis_in_pigs_as_model_for_respiratory_infections.pdf

High throughput quantitative PCR to measure prevalence and gene expression of the three major groups of treponemes associated with Digital dermatitis in dairy cows.
Objective: To develop a fast and efficient method for measuring prevalence and gene expression of representatives from the three major groups of Treponema, most frequently identified in DD biopsies from cattle.

General information
State: Published
Organisations: National Veterinary Institute, Section for Bacteriology, Pathology and Parasitology
Contributors: Schou, K. K., Weiss Nielsen, M., Jensen, T. K., Boye, M.
Number of pages: 1
Publication date: 2014
Peer-reviewed: Yes
Event: Poster session presented at Gordon Research Conferences - Unique Features of Spirochete-Host-Environment Interactions, Ventura, United States.
Electronic versions:
High-throughput screening of tick-borne pathogens in Europe

Due to increased travel, climatic, and environmental changes, the incidence of tick-borne disease in both humans and animals is increasing throughout Europe. Therefore, extended surveillance tools are desirable. To accurately screen tick-borne pathogens, a large scale epidemiological study was conducted on 7050 Ixodes ricinus nymphs collected from France, Denmark, and the Netherlands using a powerful new high-throughput approach. This advanced methodology permitted the simultaneous detection of 25 bacterial, and 12 parasitic species (including; Borrelia, Anaplasma, Ehrlichia, Rickettsia, Bartonella, Candidatus Neoehrlichia, Coxiella, Francisella, Babesia, and Theileria genus) across 94 samples. We successfully determined the prevalence of expected (Borrelia burgdorferi sensu lato, Anaplasma phagocytophilum, Rickettsia helvetica, Candidatus Neoehrlichia mikurensis, Babesia divergens, Babesia venatorum), unexpected (Borrelia miyamotoi) and rare (Bartonella henselae) pathogens in the three European countries. Moreover we detected Borrelia spielmani, Borrelia miyamotoi, Babesia divergens, and Babesia venatorum for the first time in Danish ticks. This surveillance method represents a major improvement in epidemiological studies, able to facilitate comprehensive testing of tick-borne pathogens, and which can also be customized to monitor emerging diseases.

General information
State: Published
Organisations: National Veterinary Institute, Section for Bacteriology, Pathology and Parasitology, Section for Epidemiology, Agence nationale de la sécurité sanitaire, alimentation, environnement et travail, National Veterinary Institute, Central Veterinary Institute
Number of pages: 13
Publication date: 2014
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Publication information
Journal: Frontiers in Cellular and Infection Microbiology
Volume: 4
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Article number: 103
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Ratings:
Web of Science (2019): Indexed yes
Web of Science (2018): Indexed yes
Scopus rating (2017): CiteScore 3.97 SJR 1.703 SNIP 1.097
Web of Science (2017): Impact factor 3.52
Web of Science (2017): Indexed yes
Scopus rating (2016): CiteScore 4.07 SJR 2.311 SNIP 1.305
Web of Science (2016): Impact factor 4.3
Scopus rating (2015): CiteScore 4.13 SJR 2.365 SNIP 1.406
Web of Science (2015): Impact factor 5.218
Web of Science (2015): Indexed yes
Scopus rating (2014): CiteScore 3.02 SJR 1.699 SNIP 0.998
Web of Science (2014): Impact factor 3.719
Scopus rating (2013): CiteScore 2.43 SJR 1.376 SNIP 0.3
Web of Science (2013): Impact factor 2.62
ISI indexed (2013): ISI indexed no
Scopus rating (2012): SJR 0.256 SNIP 0.189
Original language: English
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Electronic versions:
fcimb_04_00103.pdf
DOIs:
10.3389/fcimb.2014.00103
Source: PublicationPreSubmission
Source-ID: 97405789
Identification of Treponema pedis as the predominant Treponema species in porcine skin ulcers by fluorescence in situ hybridization and high-throughput sequencing.

Skin lesions often seen in pig production are of great animal welfare concern. To study the potential role of Treponema bacteria in porcine skin ulcers, we investigated the presence and distribution of these organisms in decubital shoulder ulcers (n=51) and ear necroses (n=54) by fluorescence in situ hybridization (FISH) and high-throughput sequencing. In addition, two cases of facial ulcers and five cases of other skin ulcers were included in the study. Samples from all 112 skin lesions and intact skin from pigs without skin ulcers (n=14) were screened by FISH. Three different oligonucleotide probes targeting 16S rRNA were used, specific for domain bacterium, Treponema spp. and species T. pedis. Screening showed that two cases each of facial and other ulcers, 35 (69%) of shoulder ulcers and 32 (59%) of ear necroses were positive for Treponema spp. T. pedis was the unequivocally, predominant species typically constituting more than 90% of the treponemes in a lesion, assessed visually by microscopy. Altogether, T. pedis was demonstrated in 69 of the 71 Treponema spp. positive lesions. We conclude that Treponema spp. are frequently present and abundant in various skin ulcers of pigs. The results from this study point toward an important role of T. pedis as a secondary bacterial infection in porcine skin ulcers, especially in severe and chronic lesions.

General information
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Organisations: National Veterinary Institute, Section for Bacteriology, Pathology and Parasitology, Lund University
Contributors: Karlsson, F., Schou, K. K., Jensen, T. K.
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ISSN (Print): 0378-1135
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Web of Science (2019): Indexed yes
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 2.7 SJR 1.175 SNIP 1.241
Web of Science (2017): Impact factor 2.524
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 2.65 SJR 1.363 SNIP 1.206
Web of Science (2016): Impact factor 2.628
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): CiteScore 2.56 SJR 1.413 SNIP 1.21
Web of Science (2015): Impact factor 2.564
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): CiteScore 2.54 SJR 1.291 SNIP 1.256
Web of Science (2014): Impact factor 2.511
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): CiteScore 3 SJR 1.459 SNIP 1.471
Web of Science (2013): Impact factor 2.726
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): CiteScore 3.18 SJR 1.441 SNIP 1.569
Molecular characterisation of the uterine microbiome of dairy cows suffering from endometritis, metritis, and pyometra

Postpartum uterine disease is a problem in dairy herds. Approximately 90% of dairy cows experience postpartum bacterial contamination of the uterus. Most of the cows are able to clear the infection within 8 weeks in the process of involution, but up to 20% of the cows develop metritis, which is infection throughout the uterine wall; and in some herds, 30-50% of cows develop endometritis, which is infection in the inner lining of the uterus. Pyometra is a related postpartum uterine disease, which is thought to occur when a cow with endometritis ovulates, and the cervix closes. The diseases are negatively correlated to reproductive performance, and in combination with the high incidence rate, they are costly for the farmers. Traditional culture-based studies are biased towards bacteria that thrive in a laboratory environment. In this project the bacterial flora were investigated by molecular microbiology methods, primarily 16S rRNA PCR and next generation sequencing.

The study included uterine flush samples from the lumen as well as endometrial samples, to evaluate the correlation between the uterine flush samples, which are commonly used sample type in the area, and the bacteria found adhering to
the mucosal layer of the uterus, the endometrium. It was hypothesised that pathogenic bacteria in the uterus initially adhere to the endometrium to cause disease, and that the chance of identifying pathogens is higher in examinations of endometrial biopsies than in uterine flush samples. In order to investigate the expression patterns of the bacteria in the endometritic uterus, a metatransgenomic study was performed. This method is based on mRNA sequencing, and provides a snapshot of the expression profile of the bacteria at the time of sampling. Previous studies of virulence factors have been performed with quantitative PCR, which requires prior knowledge of gene sequence.

It was found that there was an association between the *Fusobacteriaceae* and *Porphyromonadaceae* families and metritis in week 1 postpartum. For endometritis in weeks 4 and 7, there was not a bacterial family consistently associated with the disease across time points and sample types. There were large differences between the uterine flush samples and the endometrial biopsies, and although the sample types were correlated, the diversity of the microbiota in the biopsy samples was higher than the diversity of the microbiota of the uterine flush samples. Furthermore, the bacterial families that made up the majority of the population were the same over time.

The most abundant family observed in cows with pyometra was the *Fusobacteriaceae* family, which contain *F. necrophorum*, a pathogen previously known to be associated with pyometra, whereas evidence of the association of *T. pyogenes* with pyometra was less convincing. The previously unidentified Gram-negative bacteria observed in other studies of pyometra are likely to belong to the *Porphyromonadaceae*, *Pasteurellaceae*, and *Mycoplasmataceae* families identified.

It was found that the 50 most up-regulated transcripts of the microbiota from the uterus of cows with metritis and endometritis were primarily involved in DNA replication, transcription, translation, and metabolic processes. This indicates an active multiplication phase in the infection, and an adaption to the host environment. Furthermore, an up-regulation was observed of genes potentially involved in the synthesis of LPS, lipid A, haemagglutinin, and several genes that code for proteases. These genes are putative virulence genes. The majority of the most differentially expressed transcripts mapped most closely to proteins from the *F. necrophorum* and *P. levii* species. This indicates that these species were the most metabolically active in the uterus of the cows with uterine disease, and that these may be the primary pathogens of uterine disease. Transcripts from other species were also observed to be highly expressed in the uterus of cows with uterine disease, among others from *M. bovigenitalium*.

The results in this thesis underline the high number of bacteria found in the bovine postpartum uterus. *F. necrophorum* and *P. levii* were observed to be the most important pathogens in uterine disease, but the association of other bacterial species, perhaps contained within the *Mycoplasmataceae*, *Pasteurellaceae*, *Ruminococcaceae*, *Bacteroidaceae*, *Leptotrichiaceae* families, and an uncharacterised family that belong to class *Bacteroidia* seems likely. The data presented in this thesis does not support a role of the *E. coli* and *T. pyogenes* species, that have been identified as possible pathogens with traditional culture methods.

**General information**
State: Published
Organisations: National Veterinary Institute, Section for Bacteriology, Pathology and Parasitology
Contributors: Knudsen, L. R. V., Schou, K. K., Jensen, T. K., Angen, Ø.
Number of pages: 156
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Electronic versions:
PhD_Thesis_Lif_Knudsen.pdf
Research output: Research › Ph.D. thesis – Annual report year: 2015

**Multiple detection of pathogens in ticks: development of a high throughput real time PCR chip used as a new epidemiologic investigative tool**

**General information**
State: Published
Organisations: National Veterinary Institute, Section for Epidemiology, Section for Bacteriology, Pathology and Parasitology, ANSES - French Agency for Food, Environmental and Occupational Health & Safety, National Veterinary Institute, Central Veterinary Institute, National Institute of Public Health and the Environment
Publication date: 2014
Peer-reviewed: Yes

**Publication information**
Journal: Parasites & Vectors
Volume: 7
Issue number: Suppl 1
Article number: O12
The pig as a large animal model for characterization of host-pathogen interactions

Large animal models are essential in understanding the mechanisms involved in human infectious disease. To study the expression of host and bacterial genes involved in defense and survival mechanisms, we analyzed lung tissue from pigs experimentally infected with the Gram-negative bacterium A. pleuropneumoniae. All steps including RNA extraction and high-throughput real-time qPCR were carried out simultaneously for the two organisms. By applying this dual-organism approach, we obtained unique insights into the host-pathogen interaction at the site of infection. Differential expression of host genes involved in innate immune responses towards Gram-negative infections, including pattern recognition receptors and cytokines concurrent with expression of bacterial genes involved in lipopolysaccharide biosynthesis and adhesion was demonstrated.

We also studied the gene expression in blood leukocytes after experimental H1N2 virus infection of pigs, and found the regulation of several swine encoded miRNAs and cytokines to mimic key findings from influenza studies in human patients. By employing the pig as a model we were able to perform highly controlled experimental infections and to study changes of symptoms, viral titer, and expression of microRNAs/mRNAs as the influenza infection progressed in time, generating information that would be difficult to obtain from human patients.

General information
State: Published
Organisations: National Veterinary Institute, Section for Immunology and Vaccinology, Section for Bacteriology, Pathology and Parasitology
Contributors: Skovgaard, K., Brogaard, L., Heegaard, P. M. H., Schou, K. K.
Publication date: 2014
Peer-reviewed: Yes
Event: Abstract from Immunology and Infectious Diseases, Sandbjerg, Denmark.
Electronic versions:
Immunology_and_Infectious_Diseases.pdf
Source: PublicationPreSubmission
Source-ID: 100371558
Research output: Research - peer-review › Conference abstract for conference – Annual report year: 2014

Application of fluorescence in situ hybridization (FISH) for detection of bacteria in endometrial biopsies from postpartum cows

General information
State: Published
Organisations: National Veterinary Institute, Section for Bacteriology, Pathology and Parasitology, National Veterinary Institute, University of Copenhagen
Number of pages: 1
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Journal: Reproduction in Domestic Animals
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Article number: P179
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BFI (2019): BFI-level 1
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 1.56 SJR 0.594 SNIP 0.934
Web of Science (2017): Impact factor 1.422
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 1.38 SJR 0.549 SNIP 0.877
Web of Science (2016): Impact factor 1.4
Insights into the microbiota of the bovine uterus

Recent years’ advance in sequencing technology has resulted in extensive new knowledge of the microbial ecology of different environments. We used the technology to investigate the causality of endometritis, which is an inflammation in the inner lining of the uterus affecting up to 20% of dairy cows in Denmark post partum. Endometritis is linked to reduced reproductive performance, which is costly for the farmer and often leads to culling of the affected cows. With incomplete knowledge of the bacteria involved, treatment is performed without an option for choosing the best suited antimicrobial agent, which may lead to unnecessary antibiotic resistance development. Slaughterhouse samples were analysed in order to obtain information on the uterine microbiota from both cows with endometritis and seemingly healthy cows from a variety of herds.

We sampled uteri from cows (n=50) from a slaughterhouse in Holstebro, Denmark. An incision was made into the right uterine horn and an endometrial biopsy was taken with a pair of sterilised scissors. The endometrial surface was sampled with a cotton-swab through the same incision. All samples were immediately put in RNAlater. The DNA was extracted with the Maxwell 16 LEV Blood kit (Promega), the 16S rRNA PCR was performed with primers targeting the V2 region, and the 454 next generation sequencing was performed by GATC.

Previous results have shown that Proteobacteria and Tenericutes are the most important bacteria phyla in the uterus of...
Interdigital dermatitis, heel horn erosion, and digital dermatitis in 14 Norwegian dairy herds

The aim of this study was to assess infectious foot diseases, including identification and characterization of Dichelobacter nodosus and Treponema spp., in herds having problems with interdigital dermatitis (ID) and heel horn erosion (E) and in control herds expected to have few problems. We also wanted to compare diseased and healthy cows in all herds. The study included 14 dairy herds with a total of 633 cows. Eight herds had a history of ID and E, and 6 were control herds. All cows were scored for lameness, and infectious foot diseases on the hind feet were recorded after trimming. Swabs and biopsies were taken from the skin of 10 cows in each herd for bacterial analyses. In total, samples were taken from 34 cows with ID, 11 with E, 40 with both ID and E, and 8 with digital dermatitis (DD), and from 47 cows with healthy feet. Swabs were analyzed for identification and characterization of D. nodosus by PCR, culture, virulence testing, and serotyping. Biopsies were analyzed by fluorescent in situ hybridization regarding histopathology, identification, and characterization of Treponema spp., and identification of D. nodosus. Interdigital dermatitis was the most frequent foot disease, with a prevalence of 50.4% in problem herds compared with 26.8% in control herds. Heel horn erosion was recorded in 34.8% of the cows in problem herds compared with 22.1% in control herds. Dichelobacter nodosus was detected in 97.1% of the cows with ID, in 36.4% with E, in all cows with both ID and E, and in 66.0% of cows with healthy feet. All serogroups of D. nodosus except F and M were detected, and all isolates were defined as benign by the gelatin gel test. Treponema spp. were detected in 50.0% of the cows with ID, in 9.1% with E, in 67.5% with ID and E, in all cows with DD, and in 6.4% of those with healthy feet. In total, 6 previously described phylotypes (PT) of Treponema were detected: PT1, PT3, PT6, PT13, and PT15 in cows with ID, PT1 in a cow with E, and PT1, PT2, PT3, PT6, and PT13 in cows with both ID and E. One new phylotype (PT19) was identified. The epidermal damage score was higher but the difference in inflammatory response of the dermis was minor in cows with ID versus those with healthy feet. Fisher's exact test revealed an association between ID and D. nodosus, and between ID and Treponema spp. Logistic regression revealed an association between both ID and E and dirty claws (odds ratios = 1.9 and 2.0, respectively). Our study indicates that D. nodosus, Treponema spp., and hygiene are involved in the pathogenesis of ID.
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Simultaneous analysis of host and pathogen gene expression changes during bacterial infection

General information
State: Published
Organisations: National Veterinary Institute, Section for Immunology and Vaccinology, Section for Bacteriology, Pathology and Parasitology
Contributors: Brogaard, L., Hansen, M. S., Jensen, T. K., Heegaard, P. M. H., Skovgaard, K., Schou, K. K.
Publication date: 2013
Peer-reviewed: Yes
Event: Abstract from 9th Cold Spring Harbor meeting on Microbial Pathogenesis and Host Response, Cold Spring Harbor, NY, United States.
Source: dtu
Source-ID: u::9322
Research output: Research - peer-review › Conference abstract for conference – Annual report year: 2013

Targeting the Treponemal Microbiome of Digital Dermatitis Infections by High-Resolution Phylogenetic Analyses and Comparison with Fluorescent In Situ Hybridization

Modern pyrosequencing technology allows for a more comprehensive approach than traditional Sanger sequencing for elucidating the etiology of bovine digital dermatitis. We sought to describe the composition and diversity of treponemes in digital dermatitis lesions by using deep sequencing of the V3 and V4 hypervariable regions of the 16S rRNA gene coupled with species-level taxonomic identification. Treponema-specific 16S rRNA gene PCRs and pyrosequencing were performed on biopsy specimens originating from 10 different Catalan dairy herds (n = 36) with digital dermatitis, and this analysis yielded 75,297 sequences. We identified 20 different taxa, including a potentially novel phylotype that displayed 95% sequence identity to members of the Treponema denticola/Treponema pedis-like cluster. Species frequencies and abundances that were determined by pyrosequencing analysis were highly correlated with the results of fluorescent in situ hybridization using phylotype-specific oligonucleotide probes. In a limited number of animals from a single geographic region, we detected most of the Treponema phylotypes that were described in previous investigations of digital dermatitis. Additionally, we identified a number of phylotypes that mapped to oral treponemes of humans and dogs that had not been reported for digital dermatitis lesions. The results presented here support previous observations of a polytreponemal etiology of infections, with Treponema phagedenis-like, Treponema medium/Treponema vincentii-like, and T. denticola/T. pedis-like phylotypes being highly associated with disease. Using this new approach, it has become feasible to study large herds and their surrounding environments, which might provide a basis for a better understanding of the pathogenesis of this disease.

General information
State: Published
Organisations: National Veterinary Institute, Section for Bacteriology, Pathology and Parasitology, Hipra
Contributors: Schou, K. K., Foix Bretó, A., Boye, M., Jensen, T. K.
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Bovine digital dermatitis: Possible pathogenic consortium consisting of Dichelobacter nodosus and multiple Treponema species

Bovine digital dermatitis (DD) is a multifactorial disease involving at least one or more treponemal species. Virulent phytopotypes of Treponema and other infectious agents contributing to disease etiology still remain to be identified. This study addressed these questions by analyzing the prevalence and distribution of seventeen phytopotypes of Treponema in DD lesions by fluorescent in situ hybridization (FISH) applying species/phytopotype-specific oligonucleotide probes. In situ hybridization for Dichelobacter nodosus, the cause of ovine footrot, was additionally performed. We sampled 90 biopsies of DD lesions originating from one Norwegian and six Danish dairy herds, and 24 tissue samples of healthy skin. All lesions revealed intermingled infections with multiple Treponema phytopotypes (mean > 7). In six herds, the mean number of phytopotypes identified varied between 12 and 15. D. nodosus was present in forty-nine (51%) of the lesions and in three of the apparently healthy skin samples. Two “healthy” samples also contained Treponema spp. and D. nodosus, and were histopathologically categorized as subclinical DD. Another eighteen of the “healthy” skin samples showed serious epidermal hyperplasia but were not colonized by bacteria while only four samples were found normal. We hypothesise that external noxious stimuli allow D. nodosus to break down the epidermal barrier creating a suitable environment for the secondary invaders, Treponema species, which gradually take over the infection site. The variety and different distribution of treponemes in the DD lesions observed in this study, suggests that most of the Treponema phytopotypes have the potential to be pathogenic.

General information
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Organisations: National Veterinary Institute, Division of Veterinary Diagnostics and Research, Microbial Ecology, Bacteriology & Pathology, Norwegian School of Veterinary Science
Contributors: Rasmussen, M., Capion, N., Schou, K. K., Rogdo, T., Fjeldaas, T., Boye, M., Jensen, T. K.
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Scopus rating (2017): CiteScore 2.7 SJR 1.175 SNIP 1.241
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Web of Science (2017): Indexed yes
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Scopus rating (2016): CiteScore 2.65 SJR 1.363 SNIP 1.206
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Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): CiteScore 2.56 SJR 1.413 SNIP 1.21
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Web of Science (2015): Indexed yes
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Scopus rating (2014): CiteScore 2.54 SJR 1.291 SNIP 1.256
Web of Science (2014): Impact factor 2.511
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Scopus rating (2013): CiteScore 3 SJR 1.459 SNIP 1.471
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ISI indexed (2013): ISI indexed yes
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Scopus rating (2012): CiteScore 3.18 SJR 1.441 SNIP 1.569
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ISI indexed (2012): ISI indexed yes
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BFI (2011): BFI-level 2
Scopus rating (2011): CiteScore 3.27 SJR 1.56 SNIP 1.729
Web of Science (2011): Impact factor 3.327
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
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Scopus rating (2010): SJR 1.39 SNIP 1.474
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Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 1.309 SNIP 1.466
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 1.164 SNIP 1.29
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 1.048 SNIP 1.315
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 1.03 SNIP 1.396
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 1.089 SNIP 1.259
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 0.873 SNIP 1.248
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 0.905 SNIP 1.181
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 0.905 SNIP 1.13
Web of Science (2002): Indexed yes
Scopus rating (2001): SJR 0.828 SNIP 1.051
Web of Science (2001): Indexed yes
Scopus rating (2000): SJR 0.699 SNIP 1.066
Web of Science (2000): Indexed yes
Scopus rating (1999): SJR 0.714 SNIP 1.089
Investigating the etiology of bovine digital dermatitis by a combination of 16S rRNA gene analysis and fluorescence in situ hybridization

Bovine digital dermatitis, the cause of lameness and wasting in cattle, was first reported in 1974. Today, this disease has considerable negative effects on animal welfare and production economy in many parts of the world. A bacterial etiology of digital dermatitis is now well documented, and the current view on this disease points towards a complicated etiology involving co-infection of more than one, and probably multiple species belonging to the genus Treponema. Still, the pathogenic role of each of the digital dermatitis-associated phylotypes remains unclear. The aim of this investigation was to obtain a better understanding of digital dermatitis in general, including possible predisposing skin alternations and the role of the bacteria Dichelobacter nodosus. Finally, we wanted to determine if any Treponema phylotypes could be singled out as having a particularly prominent role in the etiology of the disease. Here, a PCR-based approach targeting the 16S rRNA gene along with fluorescence in situ hybridization was used to determine the prevalence and diversity of 17 Treponema phylotypes in 85 digital dermatitis lesions from six Danish dairy herds as well as additional biopsies of healthy skin and previously examined digital dermatitis lesions. All skin samples were evaluated histopathologically for possible predisposing abnormalities. Furthermore, fluorescence in situ hybridization tests for Fusobacterium necrophorum and D. nodosus was applied. All lesions revealed intermingled infections with multiple Treponema phylotypes (mean > 7). In six herds, the mean number of phylotypes identified varied between 12 and 15. D. nodosus was present in forty-nine (51%) of the lesions and in three of the apparently healthy skin samples. One "healthy" sample also contained Treponema spp. and D. nodosus, and were histopathologically categorized as subclinical digital dermatitis. We propose that external noxious stimuli allow D. nodosus to break down the epidermal barrier creating a suitable environment for the secondary invaders, Treponema species, which gradually take over the infection site. The variety and different distribution of treponemes in the digital dermatitis lesions observed in this study, suggests that most of the Treponema phylotypes have the potential to be pathogenic.

General information
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Organisations: National Veterinary Institute, Section for Bacteriology, Pathology and Parasitology
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Investigating the microbiome of the bovine uterus in relation to endometritis, a costly disease for dairy farmers
Endometritis is inflammation of the inner lining of the uterus, affecting up to 20% of the dairy cows after calving in Denmark. The disease causes reduced pregnancy rates, which often leads to culling of the cows and is costly for the farmer. Until now, investigations of which pathogens may cause the disease have been based on microbiological culturing, and no conclusive evidence has been found. Only a fraction of the bacterial flora is cultivable, and therefore more than 90% of the uterine microbiome has not been characterised. With incomplete knowledge of the pathogens, treatment is performed without an option for choosing the best suited antimicrobial agent, which may lead to unnecessary antibiotic resistance development. The present study is based on 16S rRNA PCR, which in combination with 454 next generation sequencing allows phylogenetic identification of the bacteria present in the sample. Not being limited to bacteria that are suited to growth under laboratory conditions, this study promises a more comprehensive insight into the microbiome of the dairy cow uterus than has previously been offered. Cows (n=40) on a Danish dairy herd were randomly selected on the basis of a uterine score indicating that the cows had uterine pathology. Uterine fluid was aspirated and if necessary the uterus was flushed with 30 ml sterile saline solution in order to retrieve uterine material. The fluid was placed in RNAlater. An endometrial biopsy was retrieved and the tissue placed in RNAlater. The cows were sampled on days 5-11 (week 1), days 26-32 (week 4), and on days 47-53 (week 7). This sampling schedule provided an opportunity to follow the development of any infection, and the combination of biopsy and uterine flush samples offered insights into whether tissue-invasive bacteria were present. The DNA was extracted with the Maxwell 16 LEV Blood kit (Promega), the 16S rRNA PCR was performed with primers targeting the V2 region, and the 454 next generation sequencing was performed by GATC. Previous papers based on culturing of endometrial swabs or biopsies point to Escherichia coli, Trueperella (Archanobacterium) pyogenes, and Fusobacterium necrophorum as the most likely pathogens, although some of them
also seem to be present in healthy animals. We expect to find these bacteria in the samples from the diseased animals, and perhaps the detailed data from the sequencing will also reveal hitherto undiscovered pathogens.

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Transcriptional Portrait of Actinobacillus pleuropneumoniae during Acute Disease - Potential Strategies for Survival and Persistence in the Host

**Background**
Gene expression profiles of bacteria in their natural hosts can provide novel insight into the host-pathogen interactions and molecular determinants of bacterial infections. In the present study, the transcriptional profile of the porcine lung pathogen Actinobacillus pleuropneumoniae was monitored during the acute phase of infection in its natural host.

**Methodology/Principal Findings**
Bacterial expression profiles of A. pleuropneumoniae isolated from lung lesions of 25 infected pigs were compared in samples taken 6, 12, 24 and 48 hours post experimental challenge. Within 6 hours, focal, fibrino hemorrhagic lesions could be observed in the pig lungs, indicating that A. pleuropneumoniae had managed to establish itself successfully in the host. We identified 237 differentially regulated genes likely to encode functions required by the bacteria for colonization and survival in the host. This group was dominated by genes involved in various aspects of energy metabolism, especially anaerobic respiration and carbohydrate metabolism. Remodeling of the bacterial envelope and modifications of posttranslational processing of proteins also appeared to be of importance during early infection. The results suggested that A. pleuropneumoniae is using various strategies to increase its fitness, such as applying Na+ pumps as an alternative way of gaining energy. Furthermore, the transcriptional data provided potential clues as to how A. pleuropneumoniae is able to circumvent host immune factors and survive within the hostile environment of host macrophages. This persistence within macrophages may be related to urease activity, mobilization of various stress responses and active evasion of the host defenses by cell surface sialylation.

**Conclusions/Significance**
The data presented here highlight the importance of metabolic adjustments to host conditions as virulence factors of infecting microorganisms and help to provide insight into the mechanisms behind the efficient colonization and persistence of A. pleuropneumoniae during acute disease.

**General information**
State: Published
Organisations: Division of Veterinary Diagnostics and Research, National Veterinary Institute, Microbial Ecology, National Food Institute, Division of Microbiology and Risk Assessment, Bacteriology & Pathology
Contributors: Schou, K. K., Rundsten, C. F., Jensen, T. K., Angen, Ø., Boye, M.
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Actinobacillus pleuropneumoniae transcriptome analysis during early infection - coping with a hostile environment

Aim: To obtain an increased understanding of how the porcine lung pathogen Actinobacillus pleuropneumoniae (Ap) establish infection in the host. Understanding the means by which a pathogen establishes and maintains infection in the host organism is the first step towards controlling disease. Methods: The local in vivo genetic response of Ap during the early phase of infection in porcine lungs was detailed using pangenomic microarray analysis. The global transcriptional patterns of Ap serotype 2 and 6 isolated from lung tissue biopsies of 25 experimentally infected pigs were compared at four time points between 6 and 48 hours post infection. Results: We identified 310 genes (p <1.0 × 10−8) that were differentially expressed during the first 48 hours of infection. Most of these genes appeared to be up-regulated at 6 hours post inoculation after which the expression gradually declined over the next 42 hours. Functional analysis identified a number of putative virulence genes to be initially up-regulated. Conclusions: This is the first study monitoring the development of Ap response in the porcine host during early infection. The ability of pathogenic bacteria to adjust gene expression in response to environmental stimuli is critical for bacterial survival within the host. The genes identified as differentially expressed in this study may represent a core set of genes which are mobilized to cope with the host immune response and adapt to the hostile environment. The potential virulence genes identified may represent valuable candidates for vaccine development.

General information
State: Published
Organisations: Microbial Ecology, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Division of Microbiology and Risk Assessment, National Food Institute, Bacteriology & Pathology
Contributors: Schou, K. K., Rundsten, C. F., Jensen, T. K., Angen, Ø., Boye, M.
Number of pages: 58
Pages: P52
Publication date: 2011

Comprehensive profiling of the transcriptional response to iron restriction in six serotypes of Actinobacillus pleuropneumoniae with different virulence potential

Background Comparative analysis of gene expression among serotypes within a species can provide valuable information on important differences between related genomes. For the pig lung pathogen Actinobacillus pleuropneumoniae, 15 serotypes with a considerable variation in virulence potential and immunogenicity have been identified. This serotypic diversity can only partly be explained by amount of capsule and differences in the RTX toxin genes in their genomes. Iron acquisition in vivo is an important bacterial function and in pathogenic bacteria, iron-limitation is often a signal for the induction of virulence genes. We used a pan-genomic microarray to study the transcriptional response to iron restriction in vitro in six serotypes of A. pleuropneumoniae (1, 2, 3, 5b, 6, and 7), representing at least two levels of virulence. Results In total, 45 genes were significantly (p <0.0001) up-regulated and 67 genes significantly down-regulated in response to iron limitation. Not previously observed in A. pleuropneumoniae was the up-regulation of a putative cirA-like siderophore in all six serotypes. Three genes, recently described in A. pleuropneumoniae as possibly coding for haemoglobin-haptoglobin binding proteins, displayed significant serotype related up-regulation to iron limitation. For all three genes, the expression appeared at its lowest in serotype 3, which is generally considered one of the least virulent serotypes of A. pleuropneumoniae. The three genes share homology with the hmbR haemoglobin receptor of Neisseria meningitidis, a possible virulence factor which contributes to bacterial survival in rats. Conclusions By comparative analysis of gene expression among 6 different serotypes of A. pleuropneumoniae we identified a common set of presumably essential core genes, involved in iron regulation. The results support and expand previous observations concerning the identification of new potential iron acquisition systems in A. pleuropneumoniae, showing that this bacterium has evolved several strategies for scavenging the limited iron resources of the host. The combined effect of iron-depletion and serotype proved to be modest, indicating that serotypes of both moderate and high virulence at least in vitro are reacting almost identical to iron restriction. One notable exception, however, is the haemoglobin-haptoglobin binding protein cluster which merits further investigation.

General information
State: Published
Organisations: Microbial Ecology, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Division of Microbiology and Risk Assessment, National Food Institute, Bacteriology & Pathology

Comparative profiling of the transcriptional response to iron restriction in six serotypes of Actinobacillus pleuropneumoniae with different virulence potential

Background Comparative analysis of gene expression among serotypes within a species can provide valuable information on important differences between related genomes. For the pig lung pathogen Actinobacillus pleuropneumoniae, 15 serotypes with a considerable variation in virulence potential and immunogenicity have been identified. This serotypic diversity can only partly be explained by amount of capsule and differences in the RTX toxin genes in their genomes. Iron acquisition in vivo is an important bacterial function and in pathogenic bacteria, iron-limitation is often a signal for the induction of virulence genes. We used a pan-genomic microarray to study the transcriptional response to iron restriction in vitro in six serotypes of A. pleuropneumoniae (1, 2, 3, 5b, 6, and 7), representing at least two levels of virulence. Results In total, 45 genes were significantly (p <0.0001) up-regulated and 67 genes significantly down-regulated in response to iron limitation. Not previously observed in A. pleuropneumoniae was the up-regulation of a putative cirA-like siderophore in all six serotypes. Three genes, recently described in A. pleuropneumoniae as possibly coding for haemoglobin-haptoglobin binding proteins, displayed significant serotype related up-regulation to iron limitation. For all three genes, the expression appeared at its lowest in serotype 3, which is generally considered one of the least virulent serotypes of A. pleuropneumoniae. The three genes share homology with the hmbR haemoglobin receptor of Neisseria meningitidis, a possible virulence factor which contributes to bacterial survival in rats. Conclusions By comparative analysis of gene expression among 6 different serotypes of A. pleuropneumoniae we identified a common set of presumably essential core genes, involved in iron regulation. The results support and expand previous observations concerning the identification of new potential iron acquisition systems in A. pleuropneumoniae, showing that this bacterium has evolved several strategies for scavenging the limited iron resources of the host. The combined effect of iron-depletion and serotype proved to be modest, indicating that serotypes of both moderate and high virulence at least in vitro are reacting almost identical to iron restriction. One notable exception, however, is the haemoglobin-haptoglobin binding protein cluster which merits further investigation.

General information
State: Published
Organisations: Microbial Ecology, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Division of Microbiology and Risk Assessment, National Food Institute, Bacteriology & Pathology
The effect of a diet with fructan-rich chicory roots on intestinal helminths and microbiota with special focus on Bifidobacteria and Campylobacter in piglets around weaning.

The restrictions on the use of antibiotic and anthelmintic treatments in organic pig farming necessitate alternative non-medical control strategies. Therefore, the antibiotic and parasite-reducing effect of a fructan-rich (prebiotic) diet of dried chicory was investigated in free-ranging piglets. Approximately half of 67 piglets from nine litters were experimentally infected with Ascaris suum and Trichuris suis in the suckling period (1 to 7 weeks of age) and 58 of the piglets were challenged daily with Eschericia coli O138:F8 for 9 days after weaning to induce weaning diarrhoea. The litters were fed either chicory (30% dry matter) or a control diet. The effect of chicory on intestinal helminths, intestinal microbiota, especially Bifidobacteria and Campylobacter spp. and E. coli post-weaning diarrhoea was assessed. The weight gain of the piglets was not impaired significantly by chicory. The intestinal A. suum worm burden was reduced by 64% (P = 0.034) in the chicory-fed piglets, whereas these same piglets had 63% more T. suis worms (P = 0.016). Feeding with chicory elicited no changes among the main bacterial groups in ileum according to terminal restriction fragment length polymorphism analysis. However, the terminal-restriction fragment (T-RF) 208 bp, which may belong to Lachnospiraceae, was stimulated by the chicory feed (P = 0.03), and T-RF 370 bp that matches Enterobacter belonging to the Enterobacteria was reduced (P = 0.004). In addition, chicory increased the level of Bifidobacteria (P = 0.001) and the faecal Campylobacter excretion level was transitorily reduced in chicory-fed piglets at 7 weeks of age (P = 0.029). Unfortunately, it was not possible to assess the effect of chicory on post-weaning diarrhoea as it did not develop. In conclusion, feeding piglets chicory around the time of weaning caused complex changes of the microbiota and parasite communities within the intestinal tract, and feeding piglets chicory may therefore serve as an animal-friendly strategy to control pathogens.
Comparison of high and low virulence serotypes of Actinobacillus pleuropneumoniae by quantitative real-time PCR

General information
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Expression of coding (mRNA) and non-coding (microRNA) RNA in lung tissue and blood isolated from pigs suffering from bacterial pleuropneumonia

MicroRNAs are small non-coding RNA molecules (18-23 nt), that regulate the activity of other genes at the post-transcriptional level. Recently it has become evident that microRNA plays an important role in modulating and fine tuning innate and adaptive immune responses. Still, little is known about the impact of microRNAs in the development and pathogenesis of lung infections. Expression of microRNA known to be induced by bacterial (i.e., LPS) ligands and thus supposed to play a role in the regulation of antimicrobial defence, were studied in lung tissue and in blood from pigs experimentally infected with Actinobacillus pleuropneumoniae (AP). Expression differences of mRNA and microRNA were quantified at different time points (6h, 12h, 24h, 48h PI) using reverse transcription quantitative real-time PCR (Rotor-Gene and Fluidigm). Expression profiles of miRNA in blood of seven animals were further studied using miRCURY™ LNA arrays (Exiqon, Denmark). All AP infected animals had significantly higher levels of mRNA coding for inflammatory mediators as IL-6 and IL-8 as well as the acute phase protein SAA, in the lung compared to the control group. MiR-223 was found to be highly up regulated, followed by miR-146a and to a lesser degree miR-155 in lung tissue of the AP infected animals. MiR-233 was also found to be up regulated in blood based on both microarray and real-time PCR. Mir-233 has been found to be a negative regulator of neutrophil proliferation and activation, and might act to limit the potentially harmful consequences of the accumulation of infiltrating neutrophils in AP infected lungs.

Intra-species variation in Actinobacillus pleuropneumoniae – transcriptional response to iron limitation in serotypes with different virulence potential.

Intra-species variation in Actinobacillus pleuropneumoniae – transcriptional response to iron limitation in serotypes with different virulence potential.
The porcine systemic response to pleuropneumonia studied by transcriptional profiling of liver and tracheobronchial lung lymph nodes using multiplexed mRNA-Seq

Actinobacillus pleuropneumoniae (Ap) is a gram-negative bacterium that causes porcine pleuropneumonia, which is a widespread, highly contagious and often fatal respiratory disease in swine. A total of 44 pigs were experimentally inoculated with Ap serotype 2 or 6 and samples of liver and tracheobronchial lung lymph nodes were collected 6, 12, 24 and 48 hours after experimental inoculation, as well as from six non-inoculated control pigs. Transcriptional profiles of the liver samples have been generated by preparation of 12-plexed mRNA-Seq libraries followed by sequencing on an Illumina GAIIx (51+7 cycles) obtaining more than 200 million tag sequences. The 12-plexed mRNA-Seq libraries of the lung lymph node samples have presently (April 2010) been prepared and are to be sequenced. The PCR amplicons of the liver libraries were quantified using both a fluorometer and a qPCR assay, including the use of a sequence-titrated, in-house control library. The libraries were diluted to 6 pM based on the qPCR assay, except for a single library set which was duplicated and diluted based on the fluorometer measurements as well. Analysis of the obtained sequences revealed that the qPCR based quantifications reduced the cluster density variability as compared to fluorometer based quantifications. Furthermore, it was found that the fluorometer based measurements tended to deviate for dilute as well as for more concentrated libraries. Following the sequencing of the lung lymph node samples analyses are to be conducted to study the time and serotype dependent transcriptional response to Ap infection.

Bovine digital dermatitis: A spirochetal skin disease of polytreponemal aetiology

Application of fluorescent in situ hybridisation for demonstration of multiple Treponema phylotypes involved in bovine digital dermatitis
Evidence of Multiple Treponema Phylotypes Involved in Bovine Digital Dermatitis as Shown by 16S rRNA Gene Analysis and Fluorescence In Situ Hybridization

The etiopathogenesis of the skin disease digital dermatitis (DD), an important cause of lameness in cattle, remains uncertain. Microscopically, the disease appears to be polymicrobial, with spirochetes as the predominant bacteria. The objective of this study was to identify the main part of the bacteria involved in DD lesions of cattle by using culture-independent molecular methods. Ten different phylotypes of Treponema were identified either by 16S rRNA gene sequencing of bacteria from DD lesions or by fluorescence in situ hybridization (FISH) analysis using phylotype-specific 16S rRNA-directed oligonucleotide probes. Two phylotypes, phylotype 1 (PT1) and PT2, were not closely related to any characterized treponemal species. PT7 was 99.3% identical to Treponema denticola, while PT9 resembled T. vincentii by 96%. The remaining phylotypes, PT3, PT4, PT5, PT6, and PT8, and Treponema brennaborense had previously been isolated from DD lesions. Forty DD biopsy specimens were examined for Treponema by FISH. With one exception, all of the biopsy specimens revealed epidermotropic, intermingled infection with three or more different phylotypes (mean, 4.7). The most prevalent species were PT1 (95%), PT6 (93%), and PT3 (85%). While colonization by PT3 was confined to the surface of the epidermis, both PT1 and PT6 invaded deep into the stratum spinosum and were seen in ulcerated dermal papillae. In two cases, all 10 phylotypes were demonstrated. Furthermore, FISH with a Treponema group-specific probe showed that Treponema accounted for more than 90% of the total bacterial population in the biopsy specimens. These data strongly suggest that a group of apparently symbiotic Treponema species are involved as primary bacterial pathogens in DD.

General information
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Organisations: National Veterinary Institute, Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research, University of Copenhagen
Contributors: Klitgaard, K., Boye, M., Capion, N., Jensen, T. K.
Prevalence and distribution of new and previously described Treponema phylotypes in digital dermatitis infections

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Contributors: Capion, N., Schou, K. K., Boye, M., Jensen, T. K.
Publication date: 2008
Peer-reviewed: Yes
Event: Abstract from International Symposium & the 7th Conference on Lameness in Ruminants, Kuopio, Finland.
Source: orbit
Source-ID: 231701
Research output: Research › peer-review › Conference abstract for conference – Annual report year: 2008

Etiology of digital dermatitis in Danish cattle. Preliminary results

General information
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Organisations: Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Microbial Ecology
Contributors: Jensen, T. K., Capion, N., Schou, K. K., Boye, M.
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Event: Abstract from Cattle Consultancy Days, Nyborg, Denmark.
Source: orbit
Source-ID: 241218
Research output: Research › Conference abstract for conference – Annual report year: 2007

Measurement of bacterial gene expression in vivo by laser capture microdissection and quantitative real-time RT-PCR

Due to the relative small number of bacterial pathogens present in an infected host, exploration of pathogen gene expression in vivo is challenging. This study reports the development of a protocol for quantifying bacterial gene expression in vivo in Actinobacillus pleuropneumoniae using laser capture microdissection and real-time quantitative RT-PCR.
Identification of a novel, invasive, not-yet-cultivated Treponema sp in the large intestine of pigs by PCR amplification of the 16S rRNA gene

Laser capture microdissection in combination with fluorescent in situ hybridization was used to identify an unknown species of spirochetes from the pig colonic mucosa. The 16S rRNA gene was PCR amplified, and the closest related type...
strain was Treponema bryantii(T) (90.1%). The spirochete, here named "Candidatus Treponema suis," was associated with colitis, including invasion of the surface epithelium as well as superficial parts of the mucosa.

**General information**

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Organizations: Microbial Ecology, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Section for Veterinary Diagnostics
Contributors: Mølbak, L., Schou, K. K., Jensen, T. K., Fossi, M., Boye, M.
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Peer-reviewed: Yes

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Scopus rating (2017): CiteScore 3.55 SJR 2.256 SNIP 1.443
Web of Science (2017): Impact factor 4.054
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 3.57 SJR 2.196 SNIP 1.4
Web of Science (2016): Impact factor 3.712
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 3.56 SJR 2.206 SNIP 1.431
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 3.84 SJR 2.231 SNIP 1.528
Web of Science (2014): Impact factor 3.993
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 4.18 SJR 2.438 SNIP 1.63
Web of Science (2013): Impact factor 4.232
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 4.11 SJR 2.148 SNIP 1.626
Web of Science (2012): Impact factor 4.068
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 4.27 SJR 2.346 SNIP 1.699
Web of Science (2011): Impact factor 4.153
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 2.343 SNIP 1.731
Web of Science (2010): Impact factor 4.22
Web of Science (2010): Indexed yes
Identifikation af bakterier i fikseret væv

General information
State: Published
Organisations: Microbial Ecology, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Section for Veterinary Diagnostics
Contributors: Schou, K. K., Mølbak, L., Jensen, T. K., Boye, M.
Pages: 28-29
Publication date: 2006
Peer-reviewed: Unknown

Publication information
Journal: Dansk Veterinaertidsskrift
Volume: 89
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Ratings:
BFI (2019): BFI-level 1
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
BFI (2017): BFI-level 1
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
BFI (2015): BFI-level 1
BFI (2014): BFI-level 1
BFI (2013): BFI-level 1
ISI indexed (2013): ISI indexed no
Microarray analysis of the responses of Actinobacillus pleuropneumoniae virulence genes to iron deficiency

General information
State: Published
Organisations: Microbial Ecology, Division of Veterinary Diagnostics and Research, National Veterinary Institute
Contributors: Schou, K. K., Boye, M.
Publication date: 2006
Peer-reviewed: No
Event: Abstract from 19th International Pig Veterinary Society Congress, Copenhagen, Denmark.
Source: orbit
Source-ID: 240903
Research output: Communication › Journal article – Annual report year: 2006

Phylogeny and distribution of an unknown Treponema sp. associated with porcine colitis by using in situ hybridization and laser capture microdissection (LCM)

Helical-shaped bacteria resembling Spirochaetes commonly are present in the gastrointestinal tract of animals and humans. Culturing of Spirochaetes is in general fastidious and not always successful. Here, a new DNA isolation approach for prokaryotic cells in formalin-fixed paraffin-embedded tissue samples is applied for the identification of an unknown Spirochaete. The tissue samples from colon were obtained from a previous study (1). Sectioning, in situ hybridization, LCM, DNA extraction, PCR reaction and sequencing were done according to Klitgaard et al. (2). The unknown invasive Spirochaetes species was recognized in pigs by using fluorescent in situ hybridization, followed by laser capture microdissection (LCM) of the targeted cells from the colonic mucosa. Direct 16S rRNA gene PCR was performed from the dissected micro-colonies. A phylogenetic analysis clustered the 16S rRNA gene in the Treponema genus group. The closest related Treponema type strain was T. bryantii

General information
State: Published
Organisations: Microbial Ecology, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Section for Veterinary Diagnostics
Contributors: Mølbak, L., Schou, K. K., Jensen, T. K., Fossi, M., Boye, M.
Publication date: 2006
Peer-reviewed: No
Event: Abstract from 11th International Symposium on Microbial Ecology, Vienna, Austria.
Source: orbit
Source-ID: 241063
Research output: Research › Conference abstract for conference – Annual report year: 2006

Laser capture microdissection of bacterial cells targeted by fluorescence in situ hybridization

Direct cultivation-independent sequence retrieval of unidentified bacteria from histological tissue sections has been limited by the difficulty of selectively isolating specific bacteria from a complex environment. Here, a new DNA isolation approach is presented for prokaryotic cells. By this method, a potentially pathogenic strain of the genus Brachyspira from formalin-fixed human colonic biopsies were visualized by fluorescence in situ hybridization (FISH) with a 16S rRNA-targeting oligonucleotide probe, followed by laser capture microdissection (LCM) of the targeted cells. Direct 16S rRNA gene PCR was performed from the dissected microcolonies, and the subsequent DNA sequence analysis identified the dissected bacterial cells as belonging to the Brachyspira aalborgi cluster 1. The advantage of this technique is the ability to combine the histological recognition of the specific bacteria within the tissue with molecular analysis of 16S rRNA gene or other genes of interest. This method is widely applicable for the identification of noncultivable bacteria and their gene pool from formalin-fixed paraffin-embedded tissue samples.
Laser Capture Microdissection of bacterial cells targeted by fluorescence in situ hybridization – LCM_FISH

General information
State: Published
Organisations: Microbial Ecology, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Section for Veterinary Diagnostics
Contributors: Schou, K. K., Mølbak, L., Jensen, T. K., Lindboe, C., Boye, M.
Publication date: 2005
Peer-reviewed: No
Event: Abstract from 12th European Congress on Biotechnology, Kongens Lyngby, Denmark.
Source: orbit
Source-ID: 241046
Research output: Research › Conference abstract for conference – Annual report year: 2005

Microarray analysis of the responses of Actinobacillus pleuropneumoniae virulence genes to iron deficiency

General information
State: Published
Organisations: Microbial Ecology, Division of Veterinary Diagnostics and Research, National Veterinary Institute
Contributors: Schou, K. K., Boye, M.
Publication date: 2005
Peer-reviewed: No
Event: Abstract from ASM Conference on Pasteurellaceae, Kohala Coast, HI, United States.
Source: orbit
Source-ID: 241049
Research output: Research › Conference abstract for conference – Annual report year: 2005

Projects:

Genome sequencing of important animal pathogenic bacteria
Ronco, T., PhD Student, National Veterinary Institute
Pedersen, K., Main Supervisor, National Food Institute
Schou, K. K., Examiner, National Veterinary Institute
Damborg, P. P., Examiner
Van Immerseel, F., Examiner
Stegger, M., Supervisor
Institut stipendie (DTU)
15/12/2014 → 18/04/2018
Award relations: Genome sequencing of important animal pathogenic bacteria
Project: PhD
Molecular Characterization of Endometritis in Dairy Cattle
Knudsen, L. R. V., PhD Student, National Veterinary Institute
Schou, K. K., Main Supervisor, National Veterinary Institute
Angen, Ø., Supervisor, National Veterinary Institute
Jensen, T. K., Supervisor, National Veterinary Institute
Boye, M., Examiner, National Veterinary Institute
Sheldon, I. M., Examiner
Bojesen, A. M., Examiner
Institut stipendie (DTU) Samf.
01/09/2011 → 26/01/2015
Award relations: Molecular Characterization of Endometritis in Dairy Cattle
Project: PhD

The establishment of the microbiota in piglets
Hermann-Bank, M. L., PhD Student, National Veterinary Institute
Skovgaard, K., Main Supervisor, National Veterinary Institute
Boye, M., Supervisor, National Veterinary Institute
Mølbak, L., Supervisor, National Veterinary Institute
Schou, K. K., Examiner, National Veterinary Institute
Jacobson, M., Examiner
Nielsen, D. S., Examiner
Institut stipendie (DTU) Samf.
01/07/2010 → 01/04/2015
Award relations: The establishment of the microbiota in piglets
Project: PhD

Colostrum for gut protection and recovery
Støy, A. C. F., PhD Student, National Veterinary Institute
Heegaard, P. M. H., Main Supervisor, National Veterinary Institute
Sangild, P., Supervisor
Schou, K. K., Examiner, National Veterinary Institute
Frøkiær, H., Examiner, Department of Biochemistry and Nutrition
Weström, B. R., Examiner
Institut stipendie (DTU) Samf.
01/12/2008 → 27/03/2013
Award relations: Colostrum for gut protection and recovery
Project: PhD

Bovine abortions revisited
Every month, approximately 700 bovine abortions are registered in the national Danish “Kvægdatabasen” but the number is estimated to be significantly higher as abortion-registration is deficient. Our knowledge on the causes of bovine abortion is very limited and prophylactic measures are scarce. Out of more than 100 abortion cases analysed at DTU Vet during 2014, 35% were found to have an infectious cause (bacterial infections and neosporosis). In 44% of submissions, histopathologic lesions in the placenta and/or the foetus were found, that indicate infection however, no infectious agent was detected by routine diagnostic methods. In 22% of the submitted foetuses no specific pathological findings were made. The aim of this project is to gain in-depth knowledge on the possible bacterial and viral infections of the bovine foetus and placenta by use of state of the art molecular methods for culture-independent identification of bacteria and viruses. Furthermore, placental and foetal infection is to be verified by in situ hybridization of the agents. On the basis of the project’s results, knowledge will be gained on bacterial and viral infections as causes of bovine abortions in Denmark. For example will the relevance of Chlamydia and Chlamydia-like bacteria be assessed, since those have lately been shown to play a role in swine production. The results of this study will complement knowledge-based counselling and prophylactic measures on herd-level.
Jensen, T. K., Main Supervisor, National Veterinary Institute, Section for Bacteriology, Pathology and Parasitology
Schou, K. K., Supervisor, National Veterinary Institute, Section for Bacteriology, Pathology and Parasitology
Wolf-Jäckel, G., PhD Student
01/04/2016 → 31/03/2019
Collaborators: University of Copenhagen
Project: Research