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http://www.sciencedirect.com/science/article/B6TD6-3V7WKX0-4/2/c37e5e3bbd738a17da74eeec3b6ab22d5

Based on the 16S rRNA sequences of a collection of well-characterized strains of Haemophilus somnus a set of primers was selected as candidates for a species-specific PCR test. All investigated H. somnus strains were found positive in the test, including 12 strains earlier found to represent H. somnus by DNA-DNA hybridization as well as representatives of the 16 ribotypes previously described within this species. The specificity of the test was evaluated on a broad collection of strains within the family Pasteurellaceae and on other Gram positive and negative species. None of these strains gave rise to an amplicon in the PCR test. The performance of the test on mixed cultures was evaluated by adding P. multocida to serial dilutions of H. somnus and incubating the agarplates for 1 and 2 days. This showed that the PCR test applied to the harvest from an agarplate can be expected to detect a single colony of H. somnus in the presence of 109 CFU of P. multocida even after 2 days of incubation. In conclusion, the present PCR test has been shown to represent a specific test for identification of H. somnus both in pure and mixed cultures. It represents a quick, sensitive and reliable method for identification of bacteria belonging to this phenotypically heterogeneous and often slow growing species.


http://www.sciencedirect.com/science/article/B6TD6-3WM557T-6/2/6eb8d123c9f6d4d3c70bda9878bc4e

The NADH oxidase genes (nox) of 18 strains of intestinal spirochaetes were partially sequenced over 1246 bases. Strains examined included 17 representatives from six species of the genus Serpulina, and the type strain 513AT of the human intestinal spirochaete Brachyspira aalborgi. Sequences were aligned and used to investigate phylogenetic relationships between the organisms. Nox sequence identities between strains within the genus Serpulina were within the range 86.3-100%, whilst the nox gene of B. aalborgi shared between 78.8-83.0% sequence identity with the nox sequences of the various Serpulina strains. A phenogram produced based on sequence dissimilarities was in good agreement with the current classification of species in the genus Serpulina, although an atypical strongly beta-haemolytic porcine strain (P280/1), previously thought to be S. innocens, appeared distinct from other members of this species. Primer pairs were developed from the nox sequence alignments for use in polymerase chain reaction (PCR) identification of the pathogenic species S. hyodysenteriae (NOX1), S. intermedia
(NOX2), and S. pilosicoli (NOX3), and for the combined non-pathogenic species S. innocens and S. murdochii (NOX4). The PCRs were optimised using 80 strains representing all currently described species in the genus Serpulina, as well as the type strain of B. aalborgi. Tests NOX1 and NOX4 specifically amplified DNA from all members of their respective target species, whilst tests NOX2 and NOX3 were less sensitive. NOX2 amplified DNA from all 10 strains of S. intermedia from pigs but from only 4 of 10 strains from chickens, whilst NOX3 amplified DNA from only 18 of 21 S. pilosicoli strains, even at low stringency. Tests NOX1 and NOX4 should prove useful in veterinary diagnostic laboratories, whilst NOX2 and NOX3 require further refinement.


http://www.sciencedirect.com/science/article/B6TD6-43T1NXR-1/2/502b8636708dd0db2c3da5871152271e

A single tube fluorogenic RT-PCR-based 'TaqMan' assay was developed for detection and classification of bovine viral diarrhea virus (BVDV). TaqMan-PCR was optimized to quantify BVD virus using the ABI PRISM 7700 sequence detection system and dual-labeled fluorogenic probes. Two different gene specific labeled fluorogenic probes for the 5' untranslated region (5' UTR) were used to differentiate between BVD types I and II. Sensitivity of the single tube TaqMan assay was compared with two-tube TaqMan assay and standard RT-PCR using 10-fold dilutions of RNA. Single tube TaqMan assay was 10-100-fold more sensitive than the two-tube TaqMan assay and the standardized single tube RT-PCR. Specificity of the assay was evaluated by testing different BVD virus strains and other bovine viruses. A total of 106 BVD positive and negative pooled or single serum samples, field isolates and reference strains were tested. Quantitation of cRNA from types I and II BVD virus was accomplished by a standard curve plotting cycle threshold values (CT) versus copy number. Single tube TaqMan-PCR assay was sensitive, specific and rapid for detection, quantitation and classification of BVD virus.


http://www.sciencedirect.com/science/article/B6TD6-47DD9RX-Y/2/f512e63f4f62e002c0e81e99764896d6

The pestiviruses are small enveloped RNA viruses and are causative agents of economically important animal diseases in cattle, swine, sheep and goats worldwide. We used the polymerase chain reaction to amplify one common fragment of several different strains of both hog cholera virus and bovine virus diarrhea virus (BVDV). The fragment is located at the 5'-end of the genome immediately upstream of the open reading frame. This is a highly conserved region among the different published pestivirus sequences. An internal restriction digest of the amplified fragment with Xhol and PstI was performed in order to confirm specificity of the amplified fragment. The fragment was sequenced for a number of different BVDV strains, and the sequences obtained were compared to those published and used to deduce genetic relationships between strains. Apart from this common fragment we have amplified several other fragments of the Danish BVDV strain Ug59 and obtained specific amplification fragments of the expected size.

Burr, P. D., M. E. M. Campbell, et al. (1996). "Detection of Canine Herpesvirus 1 in a wide range of
Canine herpesvirus 1 (CHV-1), a member of the alphaherpesvirus sub-family, is known to cause fatal infections in litters of puppies and may also be involved in infertility, abortion, and stillbirths in adult dogs. The purpose of this study was to determine the presence of CHV-1 DNA using the polymerase chain reaction (PCR) in twelve key sites that have been associated with latency for other herpesviruses. A 605 base pair portion of the viral glycoprotein B (gB) gene was amplified using degenerate primers, cloned, and sequenced. Conventional 20mer primers were designed using this sequence information to amplify a 120 bp fragment of gB situated between the original degenerate primers. The specificity of amplification was confirmed by Southern Blot hybridisation using an internal oligonucleotide probe. DNA was extracted from tissue samples taken from twelve dogs at post mortem and from twenty-four blood samples. Nine out of twelve dogs showed evidence of infection with CHV-1; the tissues most commonly affected were lumbo-sacral ganglia (5/12 dogs), tonsil (5/12), parotid salivary gland (4/9), and liver (4/9). No positive results were detected within the twenty-four blood samples. These results indicate that exposure to CHV-1 may be much more common than previously suggested.


A polymerase chain reaction (PCR) test for M. paratuberculosis was developed based on a 218 bp segment of a DNA insertion sequence, IS900, that is specific for this organism. The method involved two consecutive amplification reactions, with the second set of primers being nested inside the first set. The method reliably detected 50 organisms/g faeces. This PCR test was applied to 32 bovine faecal specimens containing high, moderate or low numbers of M. paratuberculosis organisms as determined by culture. The PCR test detected all specimens containing >=1600 colony forming units (cfu)/g faeces, six of ten specimens with 160-480 cfu/g faeces but only two of 13 specimens containing <= 112 cfu/g faeces. The sensitivity of this test was better than that of a commercial PCR test which was carried out on the same faecal specimens.


Clinical symptoms produced by Mycoplasma spp. and piroplasms in cats are sometimes similar. Diagnosis of these pathogens is difficult by microscopic procedures and molecular methods have been used as an alternative. We present in this work, the development of new molecular procedures for diagnosis of the aforementioned organisms, together with a molecular characterization of isolates found in southern European cats. A single PCR-RFLP procedure was designed for diagnosis of Mycoplasma spp. and a seminested PCR-RFLP was designed for
diagnosis of piroplasmids. The 16S or 18S rRNA genes of isolates found in clinical samples were partially sequenced in all positive cases. Mycoplasma spp. was detected in 9 (30%) out of 30 symptomatic cats from Spain. Sequencing indicated that 66.6% of these isolates can be ascribed to Mycoplasma haemofelis and only 33.3% to Mycoplasma haemominutum. Partial 16S rRNA sequences obtained in Spanish isolates were very similar to those previously published from the UK and the USA. The presence of piroplasmids (Babesia and Theileria spp.) was studied in 16 cats from Spain (n=13) and Portugal (n=3). Animals analyzed were 10 cats with immunosuppressive viral infection (either FeLV or FIV), 5 asymptomatic cats and 1 cat with Babesia-compatible symptoms. Asymptomatic cats were all PCR-negative. Partial sequencing of 18S rRNA gene demonstrated that the Babesia-symptomatic cat was infected with Babesia canis canis whereas 3 (30%) out of the 10 cats with immunosuppressive viral infection were coinfected with piroplasms (1 with B. canis canis, 1 with Theileria annae, and 1 with B. canis canis and T. annae both).


http://www.sciencedirect.com/science/article/B6TD6-45JPGT6-1/2/f8d6d4cdd3994a5dd14b38bc0df59594

The warm climate of Israel and mishandling of the cadavers during transit to the laboratory requires an accurate method for diagnosis of rabies in decomposed tissues. By using the reverse transcriptase polymerase chain reaction (RT-PCR) 10 decomposed brain samples that collected between 1998 and 2000 were diagnosed as negative by direct fluorescent antibody test (FAT), were found positive. Three of the 10 decomposed brains were confirmed as positive by isolation of rabies virus in tissue culture and by mouse inoculation (MIT) while the other seven decomposed samples were found positive only by RT-PCR. Direct sequencing and molecular analysis of a 328 bp fragment of the N gene of all the rabies sequences confirmed their geographical origin. These results demonstrated the importance of the RT-PCR in the detection of rabies virus in decomposed naturally infected brains, especially in cases when the sample is not suitable for other laboratory assays. Thus, the RT-PCR can provide a positive diagnosis; however, when a negative result is obtained due to the nature of the decomposed tissue that can be caused by technical reasons and a false negative might be the case.


http://www.sciencedirect.com/science/article/B6TD6-4F05RD3-1/2/d696d57ecaf39cc9e6bbff16db85461b

We describe a rapid, sensitive and reproducible real-time PCR assay for detecting and quantifying canine parvovirus type 2 (CPV-2) DNA in the feces of dogs with diarrhea. An exogenous internal control was added to control the assay performance from extraction to amplification. The method was demonstrated to be highly specific and sensitive, allowing a precise CPV-2 DNA quantitation over a range of eight orders of magnitude (from 102 to 109 copies of standard DNA). The reproducibility of the CPV-2 real-time PCR assay was assessed by calculating the coefficients of variation (CV) intra-assay and inter-assay for samples containing amounts of CPV-2 DNA spanning the whole range of the real-time PCR standard curve. Then, fecal specimens from diarrheic dogs were analyzed by hemagglutination (HA), conventional PCR and real-time amplification. Comparison between these different techniques revealed that real-time PCR is more sensitive than HA and conventional gel-based PCR, allowing to detect low viral titers of CPV-2 in infected dogs.

http://www.sciencedirect.com/science/article/B6TD6-476VM9J-191/2/df983d06b737345737f9feed4c7c98be

A polymerase chain reaction (PCR) procedure that detects proviral bovine leukaemia virus (BLV) in peripheral blood mononuclear cell DNA was evaluated. Blood samples from all animals (164) in a commercial dairy herd with a 30% prevalence of BLV infection, and from 194 animals from BLV-free herds were tested. The absence of any positive PCR results in animals from BLV-free herds confirmed the specificity of the assay. Initial testing of the infected herd using a single amplification PCR (SA-PCR), detected BLV infection in 62 of 72 adult animals that were seropositive by the agar gel immunodiffusion (AGID) test and in one persistently seronegative cow. Infection in this cow was confirmed by sheep bioassay. Subsequent testing of the SA-PCR negative, seropositive animals using a double amplification PCR (DA-PCR) detected proviral BLV in eight of nine animals that were available for retesting. The PCR assay was also able to distinguish BLV-infected calves from uninfected calves that were serologically positive because of the presence of colostral antibody. Lymphocytes from all seropositive animals were cultured for determination of BLV antigen expression. Cultures from 37 of 62 SA-PCR positive animals produced detectable quantities of viral antigens. However, antigen expression was not detected in cultures from seropositive animals that were negative in the SA-PCR. In addition, in experimental transmission tests, inoculation of more than 106 lymphocytes from these cows was required for sheep to become seropositive to BLV. These results suggest that the failure of the PCR assays to detect some seropositive animals was due to a low proportion of lymphocytes being infected with BLV in these animals. The DA-PCR detected BLV infection with a sensitivity comparable to that of the AGID test and the sheep bioassay. PCR assays may be an alternative to the sheep bioassay as an adjunct to serological testing for use in situations where it is essential to detect all infected cattle. However, the stringent precautions found to be essential to prevent false positive results due to contamination of samples with PCR product are likely to preclude the routine use of PCR for diagnosis of BLV infection.

Edmonds, M. D., A. Cloeckaert, et al. (2002). "Brucella species lacking the major outer membrane protein Omp25 are attenuated in mice and protect against Brucella melitensis and Brucella ovis." *Veterinary Microbiology* 88(3): 205.

http://www.sciencedirect.com/science/article/B6TD6-46B75TX-8/2/d776e6c53a9afc97c557eda45c3c06b5

To aid in the development of novel efficacious vaccines against brucellosis, Omp25 was examined as a potential candidate. To determine the role of Omp25 in virulence, mutants were created with Brucella abortus (BA25), Brucella melitensis (BM25), and Brucella ovis (BO25) which contain disruptions in the omp25 gene (Deltaomp25 mutants). Western immunoblot analysis and PCR verified that the Omp25 protein was not expressed and that the omp25 gene was disrupted in each strain. BALB/c mice infected with B. abortus BA25 or B. melitensis BM25 showed a significant decrease in mean CFU/spleen at 18 and 4 weeks post-infection, respectively, when compared to the virulent parental strain (Pn=5). Mice infected with B. ovis BO25 had significantly lower mean CFU/spleen counts from 1 to 8 weeks post-infection, at which point the mutant was cleared from the spleens (Pn=5). Murine vaccination with either BM25 or the current caprine vaccine B. melitensis strain Rev. 1 resulted in more than a 2 log10 reduction in bacterial load following challenge with virulent B. melitensis (Pn=5). Vaccination of mice with the B. ovis mutant resulted in clearance of the challenge strain and provided 2.5 log10 greater
protection against virulent B. ovis than vaccine strain Rev. 1. Based on these data, the B. melitensis and B. ovis [Delta]omp25 mutants are interesting vaccine candidates that are currently under study in our laboratory for their safety and efficacy in small ruminants.


http://www.sciencedirect.com/science/article/B6TD6-48XJNR6-4/2/93ee88e285f2c0b0b8ed674c6b4d1cd0

This study describes for the first time the presence of circoviruses in ostrich tissue including embryos. A polymerase chain reaction (PCR) was used for the detection of the virus in liver samples. The use of a polymerase for low copy detection significantly increased the sensitivity of the test as well as a Southern blot. Viral DNA could be detected in chicks and eggs that did not hatch. For localisation of the virus in the liver in situ hybridisation was performed on a selection of positive liver tissues.


http://www.sciencedirect.com/science/article/B6TD6-48KFGND-3/2/b03de9e7ecff9c766576b2027a19ab96

The bacterium Clostridium perfringens can cause both clinical and subclinical disease in poultry. To study the pathogenesis and epidemiology of disease caused by C. perfringens, methods for typing its various strains need to be evaluated. C. perfringens isolates from healthy and diseased poultry from different parts of Sweden were analysed by polymerase chain reaction (PCR) in order to establish the presence of [alpha]-, [beta]-, [beta]2-, [epsiv]-, [iota]- and enterotoxin genes. In order to subtype C. perfringens isolates, the two methods amplified fragment length polymorphism (AFLP) and pulsed field gel electrophoresis (PFGE) were compared on 21 C. perfringens isolates from 10 different farms. In a second study, 32 isolates of C. perfringens type A from three broilers from a healthy flock reared without ionophorous anticoccidials were subtyped by PFGE. All 53 isolates analysed with PCR belonged to the toxin type A of C. perfringens, with the gene coding for [alpha]-toxin production. Two isolates possessed the [beta]2-gene as well, but none had the other toxin genes. Both AFLP and PFGE differentiated 21 strains into 10 different subtypes. This differentiation correlated closely with the origins of the isolates. Unique subtypes were isolated from seven farms. Only isolates from birds of one farm demonstrated more than one subtype of C. perfringens. The subtyping of the isolates from a healthy flock showed that each bird carried two to three different subtypes and two different subtypes were found in the same kind of tissue sample in four cases. Three of the four different subtypes found in this study were new, compared with the first study. AFLP and PFGE were found to be equally suitable for subtyping of C. perfringens isolates. The wide variation in subtypes in the healthy broilers could be the result of the antibiotic-free rearing of these birds.

The recently described elephant endotheliotropic herpesviruses (EEHV) have been associated with the deaths of numerous captive elephants. A proposed tool for the detection of EEHV infection in elephants is the PCR-based screening for EEHV-DNA in whole blood samples. Unfortunately, this detection method has only been successful in post-mortem analyses or in animals already displaying clinical signs of EEHV disease, thus rendering this method unsuitable for identification of carrier elephants. Here, we focus on glycoprotein B (gB) for serologic assay development, since gB is an envelope protein known to induce a neutralising antibody response in other herpesvirus infections. We sequenced the entire gB gene from five Asian elephants with EEHV, representing four different gB variants. Computer-aided methods were used to predict functionally important regions within EEHVgB. An extra-cytoplasmic region of 153 amino acids was predicted to be under positive selection and may potentially contain antigenic determinants that will be useful for future serologic assay development.


Porcine postweaning Escherichia coli enteritis is a cause of significant morbidity and mortality in pigs worldwide, and effective preventive control remains an unsolved problem. This study examined the correlation between susceptibility of pigs to experimental infection with an E. coli F18 strain and the porcine intestinal F18 receptor genotypes. Thirty-one pigs classified as either belonging to the susceptible or the resistant genotype were inoculated with cultures of an E. coli O138:F18 isolated from a pig with postweaning diarrhoea. Susceptibility to colonisation and diarrhoea was assessed by clinical observations, faecal shedding of the challenge strain, histopathology and microscopic adhesion tests. Ten of 14 (71.4%) genetically susceptible pigs and one of 17 (5.9%) resistant pigs developed diarrhoea attributable to the challenge strain. There was no difference in susceptibility between homozygotic and heterozygotic susceptible pigs. Faecal shedding of the challenge strain correlated with the genetic receptor profile. Twenty pigs examined immunohistochemically revealed focal to extensive small intestinal mucosal colonisation by E. coli O138:F18 in nine of 10 susceptible and three of 10 resistant pigs. Results of in vitro adhesion assays performed with F18 cells on enterocyte preparations from 24 pigs, showed complete concordance with the F18 genotypes. In conclusion, this study showed a high correlation between the porcine intestinal F18 receptor genotypes and susceptibility to disease. However, pigs of the resistant F18 receptor genotype were not entirely protected against intestinal colonisation by E. coli F18.

isolates. In addition, SPv could be distinguished on the basis of its pathogenicity in baby mice inoculated intracerebrally. We studied the growth properties of the SPv in cell culture and its effects in a mouse respiratory and abortion model. We observed that SPv did not modify its capacity to grow in cell culture with respect to reference HH1 strain. Nevertheless, we found significant differences between the titres of the two strains at 8-14 h post-infection (PI). In this work we demonstrated that SPv showed low virulence in female at different stages of gestation, consistently, with results found in the mouse respiratory model. We considered that this low virulence of SPv could be related to its RP because the RP of HH1 strain are similar to those of the HVS25A strain and both showed effect on pregnant mice. More specific studies about genomic alterations to the SPv are necessary for identifying, more clearly, if the intra-strain variations have relation with the low virulence in the mouse respiratory and abortion model.


http://www.sciencedirect.com/science/article/B6TD6-476F474-7/2/92a4399988269b12d7d056bbb547dc5a

The native Australian marsupial Phascolarctos cinereus, otherwise known as the koala, is prone to infection by the obligate intracellular parasite Chlamydia psittaci, which causes ocular 'pink eye' and urogenital 'dirty tail' diseases. Several chlamydial DNA probes to both chromosomal and plasmid sequences were used to type by Southern blot analysis 51 samples taken from wild and captive koalas from habitats on the eastern seaboard of Australia as far apart as Queensland and Victoria. Two types of C. psittaci were observed and called types I and II. Type II was found more frequently than type I and occurred in both ocular and urogenital samples, while type I showed a strong but not absolute preference for ocular sites. Cross-hybridization analyses indicated that type I and type II had about 10% DNA sequence identity to each other. DNA analyses showed that type II was very closely related to some ovine and bovine chlamydiae but type I could not be related to any other C. psittaci strain available. Light and electron microscopic analyses of infected BGM monolayers revealed that the two strains were similar in morphological characteristics. The type I strain was considerably more infectious than the type II strain in BGM cells and in the yolk sacs of embryonated eggs. A PCR based assay detected both type I and type II koala chlamydiae in samples that had been negative by Southern blot and tissue culture and provided the first evidence that both types can occur simultaneously at the one site of infection.


http://www.sciencedirect.com/science/article/B6TD6-476TTW8-G/2/21e1d28a50e2303893510d1c51162351

Oligonucleotide primers used in a polymerase chain reaction (PCR) protocol detected the verotoxin 2 (VT2) gene in E. coli present in experimentally contaminated bull semen. The VT2 (Shiga-like toxin II [SLT-II]) primers targeted a 346-bp fragment of the gene coding for the A subunit of the toxin. PCR products, corresponding to the VT2 gene sequence, were amplified from template E. coli nucleic acid extracted from 18-h broth culture and from E. coli in contaminated semen in the undiluted state, diluted in egg yolk-Tris and diluted in milk. The sensitivity of the assay to detect E. coli was determined to be 1 pg of nucleic acid, and as few as 10-20 E. coli organisms/ml could be detected in raw and diluted semen. Preliminary confirmation of the PCR product was accomplished by slot blot hybridization to a radiolabeled specific oligoprobe. Sequencing of the PCR products identifying VT2 gene sequence revealed 99.7% homology with published gene sequences for VT2. This study demonstrates the feasibility of
applying PCR technology for the detection of E. coli in bovine semen. This technique may find wide application for the detection of other pathogens that may be present in semen.


http://www.sciencedirect.com/science/article/B6TD6-40J1DVM-5/2/f861aeb4f02e9681867e88361b77d96e

The genetic variability of a gene coding for an outer membrane lipoprotein (omlA) was used to develop a PCR typing system for Actinobacillus pleuropneumoniae. Sequence differences in the middle region of the gene divided the A. pleuropneumoniae serotypes in five distinct groups. Group I included serotypes 1, 9, 11 and 12 (omlA I), Group II consisted of serotypes 2 and 8 (omlA II), Group III included serotypes 3, 6 and 7 (omlA III), Group IV (omlA IV) consisted of serotype 4 and Group V of serotypes 5a, 5b and 10 (omlA V). The sequence differences were utilized to construct PCR primers specific for each group, except of Group IV, as the amplicon of serotype 4 could be separated from Group III by size. Together with a PCR apx typing system, the omlA PCR typing system could discriminate the majority of A. pleuropneumoniae serotypes of biovar 1 except of serotypes 1, 9 and 11 and serotypes 2 and 8. The PCR typing system was tested on 102 field strains of A. pleuropneumoniae isolated from lungs of diseased pigs. The serotyping results of the investigated field strains were in agreement with the apx and omlA gene patterns found in the reference strains of the bacteria, with the exception of the omlA gene of five strains of serotype 8. To examine the apx and omlA gene pattern of tonsil isolates, the PCR typing system was tested on a total of 280 A. pleuropneumoniae field strains isolated from tonsils of pigs. Agreement between serotyping and DNA typing was found in 96% of the isolates using the apx gene patterns and in 89% of the isolates using the omlA gene. The same serotype specific apx/omlA gene pattern was thus found in the majority of the tonsil isolates and in isolates from diseased lungs. Most of the differences in the omlA gene were found in 18 tonsil isolates of serotype 12. The omlA/apx PCR typing system described in the present study makes it possible to determine the type specificity of the majority of A. pleuropneumoniae isolates by simple PCR technique and enables phenotype independent characterization of isolates non-typable by serotyping.


http://www.sciencedirect.com/science/article/B6TD6-3W0FBDG-B/2/d92518ae253c6f1038f7707ca3438b04

A PCR for the detection of Actinobacillus pleuropneumoniae was evaluated. All of 102 field isolates of A. pleuropneumoniae reacted in the PCR by amplification of a 985 bp product. No PCR amplification product was observed when examining strains of A. wreae, A. capsulatus, A. hominis, A. equuli, A. rossii, A. suis, Escherichia coli, Bordetella bronchiseptica, Streptococcus suis, Pasteurella haemolytica, Pasteurella multocida, Haemophilus parasuis, Haemophilus taxon Minor group, Haemophilus taxon D/E and Haemophilus taxon F. Amplification of a 985 bp product was, however, observed when testing strains of A. lignieresii. The lower detection limit of the PCR test was 103 A. pleuropneumoniae CFU/PCR test tube and was not affected by addition of 106 E. coli CFU/PCR test tube. Mixed bacterial cultures from tonsils of 101 pigs from 9 different herds were tested by culture and by PCR using four different bacteriological media. While 65% reacted positive in the PCR only 23% were positive by culture, thereby suggesting a superior sensitivity of the PCR test to that of culture. The use of selective media, large inoculum and incubation for 48 h
The highest number of positive PCR reactions from mixed bacterial cultures. Tonsil cultures from 50 pigs from an A. pleuropneumoniae-negative herd did not react in the PCR. The results show that PCR on mixed bacterial cultures from tonsils may be a highly sensitive method for the detection of A. pleuropneumoniae in pig herds.


A Slovenian BVD control and eradication programme was initiated in 1994, and the results from testing of bovine herds for antigen and antibodies in 1996 are presented. Samples originating from breeding herds, breeding herds for young bulls, and insemination stations were tested by antigen or antibody ELISA, or by PCR. Out of 7968 samples from 354 herds we found 18% of the animals antibody-positive. In one region situated in the north-east of Slovenia we found the herds to be almost nearly free of BVDV infections (5% prevalence). No positive antigen ELISA findings were done in 374 blood samples from recruitment herds for young bulls, whereas two out of 206 sera were investigated by PCR-reacted positive. The differences in seroprevalence found between regions is thought to be caused by differences in summer pasturing and husbandry practices.


The family Herpesviridae is a large group of viruses which contain double stranded DNA genomes. Biological characteristics, such as host signs, site of replication and site of latency have been used to describe three major subfamilies, Alphaherpesvirinae, Betaherpesvirinae and Gammaherpesvirinae within the family Herpesviridae. Macropodid herpesviruses (MaHV) have been implicated in fatal outbreaks amongst the captive marsupial populations of Australia. These outbreaks have resulted in the isolation of nine MaHV strains which have been classified into two species called macropodid herpesvirus 1 and 2 (MaHV-1 and MaHV-2). Biological characteristics have been used to place MaHV-1 and -2 within the subfamily Alphaherpesvirinae. Molecular phylogenetic reconstructions indicate an unusual position for MaHV-1 and -2 within the alphaherpesviruses. Current isolates of MaHVs have all been obtained from marsupials exhibiting clinical disease. A common biological characteristic of herpesviruses is the establishment of latent infections in nervous tissue. We have determined that MaHV are able to latently infect eastern grey kangaroos through reactivating and isolating a herpesvirus by inducing immunosuppression. We have investigated the possible sites of latency for MaHV-1 using molecular techniques. Detection of herpesvirus DNA in the trigeminal ganglia taken from two naturally infected eastern grey kangaroos indicates dissemination via a respiratory route.

A reverse transcriptase-polymerase chain reaction (RT-PCR) assay for the detection of the feline coronavirus (FCoV) genome and a co-cultivation method for the isolation of field strains of FCoV are described. Using the RT-PCR assay to assess blood samples from cats with feline infectious peritonitis (FIP) (n=47) and healthy cats from households with endemic FCoV (n=69) it was shown that approximately 80% of the cats were viraemic, irrespective of their health status. It was also shown that, over a 12-month period, a similar percentage of healthy cats remained viraemic, and that the presence of viraemia did not appear to predispose the cats to the development of FIP. The co-cultivation system proved to be a suitable method for the culture of field strains of FCoV from blood samples, so long as the cultures were maintained for at least 4 weeks. Using this system, followed by the RT-PCR, viraemia was detected as frequently as by RT-PCR on RNA extracted directly from peripheral blood mononuclear cells.


For 79 isolates from the tonsils of healthy cattle identified as Erysipelothrix by cultivation, biochemical and serological tests, genotypic identification was performed by polymerase chain reaction (PCR) using four species-specific sets of oligonucleotide primers (ER1F-ER1R, ER2F-ER2R, ER3F-ER3R and ER4F-ER4R). The results of PCR for 79 bovine isolates were compared with those of serological typing. For 19 isolates, serotyping and genotyping results were the same. PCR allowed for the identification of 36 untypable isolates as Erysipelothrix species, strain 1. Serotyping and genotyping results of the remaining 24 isolates were different. Supplemental tests are frequently needed for Erysipelothrix identification.


A polymerase chain reaction (PCR) assay was developed to detect Chlamydia psittaci DNA in faeces and tissue samples from avian species. Primers were designed to amplify a 264 bp product derived from part of the 5’ non-translated region and part of the coding region of the ompA gene which encodes the major outer membrane protein. Amplified sequences were confirmed by Southern hybridization using an internal probe. The sensitivity of the combined assay was found to be between 60 to 600 fg of chlamydial DNA (approximately 6 to 60 genome copies). The specificity of the assay was confirmed since PCR product was not obtained from samples containing several serotypes of C. trachomatis, strains of C. pneumoniae, the type strain of C. pecorum, nor from samples containing microorganisms commonly found in the avian gut flora. In this study, 404 avian faeces and 141 avian tissue samples received by the Central Veterinary Laboratory over a 6 month period were analysed by PCR, antigen detection ELISA and where possible, cell culture isolation. PCR performed favourably compared with ELISA and cell culture, or with ELISA alone. The PCR assay was especially suited to the detection of C. psittaci DNA in avian faeces samples. The test was also useful when applied to tissue samples from small contact birds associated with a case of human psittacosis where ELISA results were
negative and chlamydial isolation was a less favourable method due to the need for rapid
diagnosis.

Hirasawa, T., T. Kaneshige, et al. (1994). "Sensitive detection of canine parvovirus DNA by the nested

http://www.sciencedirect.com/science/article/B6TD6-476VK43-TY/2/e43e77c6eb336358e0314272f80dad6f

A polymerase chain reaction (PCR) for the detection of canine parvovirus (CPV) was developed.
To increase the sensitivity and specificity of the reaction, the nested PCR with a double-nested
primer pair (inner primer pair) was designed. The sequences of the PCR primer pairs were
selected from the conserved region in the CPV VP1/VP2 gene. The PCR with the outer or inner
primer pair alone (single PCR) could detect 10 fg of viral replicative form (RF) DNA on agarose
gel electrophoresis; whereas as little as 100 ag of the RF DNA was detected by the nested PCR,
which was shown to be 100 times more sensitive than the single PCR. Samples prepared from
feline panleukopenia virus and mink enteritis virus, both having a very close antigenic relationship
to CPV, were also amplified by the nested PCR. The specificity of the reaction was confirmed by
restriction enzyme analysis and Southern hybridization. Next, fecal samples were examined by
the nested PCR. All 10 samples suspected of CPV infection were positive, and two restriction
sites (HaeIII and HindIII sites) in the PCR product were conserved among them. On the other
hand, specific amplification was not observed in the samples derived from normal dogs. The
number of the genome copy in positive samples was estimated about 109-1011/g by the single
PCR and 1011-1013/g by the nested PCR. The assay can be completed in 1-1.5 days, and does
not need radioisotopes. Thus, the nested PCR seems to be a sensitive, specific and practical
method for the detection of CPV in fecal samples.

Huang, B., S. Subramaniam, et al. (2002). "Vaccination of ducks with recombinant outer membrane
protein (OmpA) and a 41 kDa partial protein (P45N') of Riemerella anatipestifer." *Veterinary
Microbiology* 84(3): 219.

http://www.sciencedirect.com/science/article/B6TD6-449V2NF-
1/2/e08033c518bf236da7cd8900f8e827d8

The generation of protective immunity against Riemerella anatipestifer infection in ducks were
investigated by immunizations with recombinant glutathione sulfatransferase (GST) fusion's
proteins of OmpA, a 42 kDa major outer membrane protein, and P45N', a 41 kDa N-terminal
fragment of a newly identified 45 kDa potential surface protein from R. anatipestifer. The DNA
encoding OmpA and P45N' were isolated from R. anatipestifer serotype 15 (field strain 110/89)
and serotype 19 (reference strain 30/90), respectively. Immunoblotting and ELISA results showed
that the purified recombinant proteins induced the production of antibodies in immunized ducks.
However, neither was protective against subsequent challenge with the virulent serotype 15
strain, 34/90. All the five ducks immunized with formalinized R. anatipestifer strain 34/90 survived
the challenge with the homologous strain whereas six out of seven ducks in the non-immunized
control group died within a week following the challenge.

isolated from pigs with oedema disease or postweaning diarrhoea." *Veterinary Microbiology* 40(3-
The study comprises fifty 4 to 12 weeks old pigs that died from oedema disease or severe diarrhoea. Smears were prepared from the mucosa of duodenum, jejunum and ileum, and by immunofluorescence F107 fimbrial antigens were detected. E. coli. strains were isolated from the intestines and were characterised by slide agglutination (serogroup and F107 fimbriae production), by their cytotoxicity for Vero cells, and by gene amplification (genes coding for the major F107 subunit FedA, the toxin causing oedema disease SLT-IIv, and enterotoxins LT1, ST1a and ST1l). F107 fimbriae were demonstrated in association with E. coli of serogroups O139:K12 and O141:K85a,b but not of serogroup O149:K91:F4a,c. Expression in culture of F107 fimbriae by some isolates gave additional evidence for production of these fimbriae by ETEC strains. The genetic determinant of SLT-IIv was found in association with F107, and could not be detected in serogroup O149:K91:F4a,c. Gene fedA was demonstrated in two isolates which were devoid of SLT-IIv. Most isolates from cases of oedema disease belonged to serogroup O139:K12 and did not contain enterotoxin genes. Isolates from pigs that suffered from diarrhoea were serotyped O141:K85a,b or O149:K91:F4a,c, and carried at least two enterotoxin genes in their genomes. In a small proportion of the cases F107 antigens were demonstrated in intestinal smears although gene fedA was not detected in the corresponding isolates. The results confirm the importance of F107 fimbriae as virulence factor in oedema disease E coli strains, but also demonstrate that F107 fimbriae can be found in association with postweaning diarrhoea isolates. In these latter strains enterotoxins were always demonstrated, irrespective of the presence of toxin SLT-IIv.

Jacobson, M., A. Aspan, et al. (2004). "Routine diagnostics of Lawsonia intracellularis performed by PCR, serological and post mortem examination, with special emphasis on sample preparation methods for PCR." Veterinary Microbiology 102(3-4): 189.

The aim of this study was to find suitable and reliable tools for demonstrating Lawsonia intracellularis in routine clinical diagnosis. Firstly, a method to prepare tissue samples before a polymerase chain reaction (PCR) was evaluated in pigs submitted for necropsy. Secondly, seven different faecal preparation methods and four different DNA polymerases were tested in single or nested PCR, with co-amplification of a mimic molecule. Thirdly, in selected pigs submitted for necropsy, tissue and faecal samples were examined histopathologically and by PCR, and blood samples were analysed serologically. Detection of L. intracellularis in tissue preparations by PCR showed good specificity and correlated to lesions found at necropsy. The sensitivity in spiked tissue samples was 101-102 mimic molecules per tube. In faecal samples, nested PCR on boiled lysate gave the best result with a sensitivity of 102-103 mimic molecules per reaction tube. However, because of the time-consuming procedure and the increased risk for contamination, a commercially available kit was preferred for routine diagnoses, despite a somewhat lower detection rate in subclinically infected pigs. In a few cases, the serological results differed from those obtained by PCR and by necropsy but the reason for this is not clear. This study indicates that the best method for diagnosis of acute enteritis in growers is PCR on faecal or tissue samples. To determine the presence of the bacteria in a herd, serology or repeated faecal sampling for PCR from target animals, or both, should be used.

This study was undertaken to determine the in vitro susceptibility of Clostridium perfringens, isolated from poultry to antimicrobials used in poultry production. The minimal inhibitory concentration (MIC) of eight antimicrobials, including the ionophoric coccidiostat narasin, was determined for 102 C. perfringens isolates, 58 from Sweden, 24 from Norway and 20 from Denmark. Susceptibility to each antimicrobial compound was determined by broth microdilution. The isolates were obtained from broilers (89), laying hens (9) and turkeys (4), affected by necrotic enteritis (NE) or by C. perfringens associated hepatitis (CPH), and from healthy broilers. All strains, regardless of origin, proved inherently susceptible to ampicillin, narasin, avilamycin, erythromycin and vancomycin. A low frequency of resistance to virginiamycin and bacitracin was also found. Resistance to tetracycline was found in strains isolated in all three countries; Sweden (76%), Denmark (10%) and Norway (29%). In 80% of the tetracycline-resistant isolates, the two resistance genes tetA(P) and tetB(P) were amplified by PCR whereas in 20% only the tetA(P) gene was detected. No tetM gene amplicon was obtained from any of the tetracycline-resistant isolates. The uniform susceptibility to narasin revealed in this study shows that the substance can still be used to control clostridiosis. In this study, C. perfringens also showed a low degree of resistance to most other antimicrobials tested. Despite the small amounts of tetracycline used in poultry, a considerable degree of resistance to tetracycline was found in C. perfringens isolates from Swedish broilers.


Transmission of Lawsonia intracellularis from experimentally inoculated pigs to naive swine was demonstrated in this study. The study was conducted using conventional pigs divided into three groups as follows: principles inoculated with L. intracellularis, sentinels, and controls. The pigs were inoculated and paired on 13 and 9 days post-inoculation with a sentinel pig for 7 days. Fecal samples and serum samples were collected throughout the study for polymerase chain reaction (PCR) and antibody testing by indirect fluorescent antibody techniques. After co-mingling, the inoculated group was necropsied; sentinel and control pigs were necropsied 7-14 days later. The intestinal tracts were evaluated grossly and microscopically for lesions. PCR was performed on intestinal mucosal scrapings and feces. Warthin-Starry and fluorescent antibody staining procedures were conducted to confirm colonization with L. intracellularis. Gross and microscopic lesions typical of porcine proliferative enteropathy (PPE) were observed in both the inoculated and sentinel groups. Transmission was demonstrated from inoculated principle pigs to sentinel pigs. PCR results detected cyclical shedding of L. intracellularis in the feces. Seroconversion occurred in pigs that were exposed to L. intracellularis. From this study, it was demonstrated that transmission of L. intracellularis can occur easily in an environment with experimentally infected pigs and that PCR can be a useful tool to monitor fecal shedding of the organism.

Bordetella bronchiseptica is a respiratory tract pathogen in a variety of species. Previous studies suggest little genetic variation among canine B. bronchiseptica isolates. The degree of genetic diversity in 26 canine B. bronchiseptica strains was evaluated using randomly amplified polymorphic DNA (RAPD) fingerprinting and ribotyping. Strains evaluated include historic reference strains (N = 3), vaccine strains (N = 5) and clinical isolates (N = 18). RAPD fingerprinting with the 10-nucleotide primer OPA-4 resulted in four distinct fingerprint patterns. RAPD fingerprinting consistently separated four previously characterized electromorphotype (EMT) 6 strains into two fingerprint types. Ribotyping, using the restriction endonuclease PvuI, resulted in six distinct ribotypes. With the exception of vaccine strains, considerable genetic diversity exists in the canine B. bronchiseptica isolates examined. These findings indicate the genetic variability within canine strains of B. bronchiseptica is greater than appreciated previously. Additionally, OPA-4 RAPD fingerprinting and PvuI ribotyping will be useful tools in epidemiologic studies of canine B. bronchiseptica isolates.


http://www.sciencedirect.com/science/article/B6TD6-441H24T-5/2/eece5601046707d9b4fab5af2f29b98e136

Swine herds in the US have experienced recent outbreaks of a severe form of porcine reproductive and respiratory syndrome (designated acute or atypical PRRS) characterized by abortion and high mortality in pregnant sows. Most of the affected herds had been vaccinated with modified live-vaccines (MLVs) against PRRS. To explore the possible mechanism of the emergence of acute PRRS, the open reading frame 5 (ORF5) gene encoding the major envelope protein (GP5) of acute PRRSV isolates was characterized. The complete ORF5 gene of eight acute PRRSV isolates from herds experiencing acute PRRS outbreaks in Iowa and North Carolina was amplified and sequenced. Sequence analyses revealed that these acute PRRSV isolates shared 88-95% nucleotide and 88-96% amino acid sequence identities to each other, 87-97% nucleotide and 84-96% amino acid sequence identities with other North American PRRSV isolates and the MLVs. Most of the amino acid substitutions locate in the putative signal sequence and two short hypervariable regions at the amino terminus. The ORF5 gene sequence of the acute PRRSV isolate 98-37120-2 from a non-vaccinated swine herd in Iowa is very closely related to that of the RespPRRS MLV, with 97% nucleotide and 96% amino acid sequence identities. Phylogenetic analysis revealed that all eight acute PRRSV isolates are clustered within the North American genotype. Several minor branches that are not associated with geographic origins were also identified within the North American genotype. One acute PRRSV isolate (98-37120-2) is clustered with the RespPRRS MLV and several Danish isolates that were confirmed to be derived from the RespPRRS MLV. The ORF5 gene sequences of other seven acute isolates are more related to those of several earlier PRRSV isolates and the PrimePac MLV than to that of the RespPRRS MLV. Our results showed that the acute PRRSV isolates analyzed in this study differed from each other in ORF5 genes, although they all clustered within the North American genotype. The data from this study do not fully support the hypothesis that the emergence of acute PRRS is due to reversion of MLVs to a pathogenic phenotype, as only one of the eight acute isolates was shown to be very closely related to the RespPRRS MLV.


http://www.sciencedirect.com/science/article/B6TD6-4325YY9-
The study describes for the first time the phylogenetic relationship between equine arteritis virus (EAV) isolated from asymptomatic virus-shedding stallions and fatal cases of equine viral arteritis (EVA) in an European country. EAV was isolated from three dead foals and an aborted foetus during three different outbreaks of EVA. From these fatalities, the complete open reading frame 5, encoding the EAV GL protein, was amplified by reverse transcription-polymerase chain reaction and subjected to nucleotide sequence analysis. Furthermore, DNA sequences were obtained from virus isolated from semen samples of seven virus-shedding, but clinically healthy, Danish stallions. DNA sequence alignment revealed an overall divergence of 0-14 and 0-10% at the nucleotide and amino acid levels, respectively. Phylogenetic analysis including 24 previously published sequences revealed that European as well as North American "types" of EAV were present in the semen of asymptomatic carrier stallions and in fatal cases of EVA. Our results reveal that the presence of EAV-shedding stallions in Denmark represents a potential source of severe EVA.


http://www.sciencedirect.com/science/article/B6TD6-3TTCH9V-2/2/b4ce58f358a07f71bcf65c8d2c643

Danish isolates of bovine respiratory syncytial virus (BRSV) were characterised by nucleotide sequencing of the G glycoprotein and by their reactivity with a panel of monoclonal antibodies (MAbs). Among the six Danish isolates, the overall sequence divergence ranged between 0 and 3% at the nucleotide level and between 0 and 5% at the amino acid level. Sequence divergences of 7-8%, 8-9% and 2-3% (nucleotide) and 9-11%, 12-16% and 4-6% (amino acid) were obtained in the comparison made between the group of Danish isolates and the previously sequenced 391-2USA, 127UK and 220-69Bel isolates, respectively. Phylogenetic analysis showed that the Danish isolates formed three lineages within a separate branch of the phylogenetic tree. Nevertheless, the Danish isolates were closely related to the 220-69Bel isolate, the prototype of the intermediate antigenic subgroup. The sequencing of the extracellular part of the G gene of additional 11 field BRSV viruses, processed directly from lung samples without prior adaption to cell culture growth, revealed sequence variabilities in the range obtained with the propagated virus. In addition, several passages in cell culture and in calves had no major impact on the nucleotide sequence of the G protein. These findings indicated that the previously established variabilities of the G protein of RS virus isolates were not attributable to mutations induced during the propagation of the virus. The reactivity of the Danish isolates with G protein-specific MAbs were similar to that of the 220-69Bel isolate. Furthermore, the sequence of the immunodominant region was completely conserved among the Danish isolates on one side and the 220-69Bel isolate on the other. When combined, these data strongly suggested that the Danish isolates belong to the intermediate subgroup.


http://www.sciencedirect.com/science/article/B6TD6-40J1DVM-7/2/425b31a9a2087b48989a772126cdc392
Fifty six Danish streptomycin (Sm) resistant isolates of Salmonella enterica serotype Typhimurium from pigs (n=34), calves (n=3) and humans (n=19) were characterised with respect to co-resistances (14 drugs), transferability of Sm-resistance by conjugation, genetic determinants encoding Sm-resistance and diversity with respect to localisation of genes in the genome and DNA-sequences. Forty-six strains carried resistance(s) other than Sm-resistance. Nineteen different co-resistance patterns were observed and tetracycline was the most commonly observed resistance in these patterns. In 22 of the strains, Sm-resistance was transferred by conjugation. Eleven strains contained the gene aadA only, six strains contained aadA+strA+strB, and 35 strains contained strA+strB. Partial sequences of aadA were obtained from four strains. Three strains showed identical sequences to a published aadA sequence from the transposon Tn7, and in one strain the sequence showed one synonymous substitution compared to this sequence. Partial sequences were obtained of strA and strB in seven strains. The sequence of strB was identical to the published sequence of the plasmid RSF1010 in all strains. All seven sequences of strA were identical and differed from the sequence of strA in RSF1010 by two non-synonymous substitutions.


http://www.sciencedirect.com/science/article/B6TD6-4FBFPYW-2/2/9e72adbc4ab379211e19c5a081d4368c

Eighty-nine bovine viral diarrhoea viruses (BVDV) from Australia have been genetically typed by sequencing of the 5’ untranslated region (5’-UTR) and for selected isolates the Npro region of the viral genome. Phylogenetic reconstructions indicated that all of the samples examined clustered within the BVDV type 1 genotype. Of the 11 previously described genetic groups of BVDV-1, 87 of the samples examined in this study clustered with the BVDV-1c, while two samples clustered with the BVDV-1a. Based on these analyses there appears to be limited genetic variation within the Australian BVDV field isolates. In addition, the phylogenetic reconstructions indicate that the clustering of Australian BVDV in the phylogenetic trees is not a result of geographic isolation.


http://www.sciencedirect.com/science/article/B6TD6-405KDD3-6/2/113e05879cefaa770960b824e3b5dd7

The amino acid sequences of the HA1 portion of the haemagglutinin of two equine A(H3N8) influenza viruses isolated in France in 1993 and 1998 were analysed to determine their evolutionary relationship with 51 other HA1 amino acid sequences available in databanks. Our data show that the French strain isolated in 1993 belongs to a group of phylogenetically related viruses branched on the main trunk, illustrating the main lineage of evolution of the equine-2 H3 sequences before its split into two distinct lineages in the late 1980s. By contrast, the 1998 French isolate appears to belong to the more recent 'Eurasian' lineage. These data suggest that equine-2 strains antigenically related to old prototype viruses may cocirculate with the more recent 'Eurasian' and 'American' lineages. In conclusion, it may be necessary to include both strains representative of recent equine influenza variants and an older prototype strain in the current equine influenza vaccines.
Human TT virus (TTV), originally isolated from a patient with post-transfusion hepatitis in 1997, is ubiquitous and non-pathogenic. Viruses related to human TTV have since been identified in non-human primates, bovine, ovine, porcine, feline, and canine. The objective of this study was to genetically characterize porcine TTV from pigs in different geographic regions. PCR primers based on the non-coding region of the only available porcine TTV isolate were designed to amplify porcine TTV DNA from sera of pigs in six different countries. Porcine TTV DNA was detected in 66.2% (102/154) of the swine sera. The percentages of positive pigs varied greatly from country to country and even within the same country: 33% in Iowa, USA; 40% in Thailand; 46% in Ontario, Canada; 80% in China; 85% in Korea; 90% in Spain; 100% in Quebec and Saskatchewan, Canada. A total of 40 porcine TTV isolates (five from each geographic region) were sequenced for a 218 bp fragment within the non-coding region. Sequence analyses revealed that porcine TTV isolates from different geographic regions shared 86-100% nucleotide sequence identity to each other. The prototype Japanese isolate of porcine TTV, Sd-TTV31, shared 90-97% nucleotide sequence identity with porcine TTV isolates reported in this study. Phylogenetic analysis showed that the clustering of the porcine TTV isolates is not associated with geographic origins. Although porcine TTV is not known to be associated with any swine disease, co-infection of pigs with TTV and other known swine pathogens may result in enhanced disease. There are also concerns for risk of potential human infection during xenotransplantation.


In the US eradication program for bovine tuberculosis, a definitive diagnosis depends on the isolation of Mycobacterium bovis. However, in some cases bacterial culture is unsuccessful, even though the tissue is considered suspicious by histopathology because granulomatous lesions and acid-fast organisms are present. The purpose of this study was to determine if polymerase chain reaction (PCR) tests on formalin-fixed tissue would successfully identify the organisms observed in suspect lesions from culture-negative animals. Diagnostic laboratory records were used to select paraffin blocks of tissue from 102 ruminants that had suspect microscopic lesions but no bacterial isolation. Sections from these blocks were examined with PCR primers for IS6110 to detect Mycobacterium tuberculosis complex infection, or with 16S ribosomal RNA and IS900 primers for detection of Mycobacterium avium. The PCR tests successfully identified a mycobacterial infection in 58 of 102 tissues, including 41 M. tuberculosis complex and 17 M. avium (11 subspecies paratuberculosis). These results demonstrate that PCR testing of formalin-fixed tissue, in combination with bacterial culture, may increase the effectiveness of laboratory diagnostic efforts to detect and identify the most common mycobacterial diseases of ruminants.

Canine parvovirus type 2 (CPV-2) and feline panleukopenia (FPL) virus (FPLV) are well known and ubiquitous diarrhea-causing pantropic viruses. A "new" antigenic variant of CPV-2 (designated as CPV-2a) has been also prevalent among dogs in Japan. In the present study, 24 canine and 8 feline isolates collected during 1987-1991 were compared with 17 CPV-2 or CPV-2a and 7 FPLV strains that had been characterized previously. Genomic properties were determined by the restriction cleavage patterns of amplified genes encoding the capsid proteins VP1 and VP2 by the polymerase chain reaction. Antigenic properties were determined by hemagglutination-inhibition assay with monoclonal antibodies against an FPLV strain. Growth characteristics in feline CRFK and canine MDCK cells were also examined. Genomic and antigenic properties of the canine isolates were relatively invariable with one exceptional isolate, C27, which was recovered from a typical clinical case of parvovirus infection but possessed properties similar to FPLV rather than CPV-2 and CPV-2a. All isolates from FPL cases possessed the same genomic and antigenic properties as those of reference FPLVs isolated in the 1970s, but three of five strains isolated from the feces of clinically healthy cats were likely to be of canine origin because they possessed very similar properties to CPV-2a. Although species-specificity of these novel isolates could not be determined definitely, the results indicate a possibility that transmission of parvovirus has occurred between these two animal species.

Bartonella species are emerging pathogens that have been isolated worldwide from humans and other mammals. Our objective was to estimate the prevalence of Bartonella infection in free-ranging African lions (Panthera leo) and cheetahs (Acinonyx jubatus). Blood and/or serum samples were collected from a convenience sample of 113 lions and 74 cheetahs captured in Africa between 1982 and 2002. Whole blood samples available from 58 of the lions and 17 of the cheetahs were cultured for evidence of Bartonella spp., and whole blood from 54 of the 58 lions and 73 of the 74 cheetahs tested for the presence of Bartonella DNA by TaqMan PCR.

The prevalence of seropositivity, bacteremia, and positive TaqMan PCR was not significantly different between sexes and age categories (juvenile versus adult) for both lions and cheetahs. Domestic cats are thus no longer the only known carriers of Bartonella spp. in Africa. Translocation of B. henselae seronegative and TaqMan PCR negative wild felids might be effective in limiting the spread of Bartonella infection.
The aim of this study was to analyse the genetic diversity among Clostridium perfringens isolates from Danish broiler chickens since both sick and presumably healthy animals were investigated. Isolates (n=279) collected from chickens from 25 farms were analysed by pulsed-field gel electrophoresis (PFGE) with the restriction enzyme SmaI. A high genetic diversity was found. Isolates with different PFGE types were toxin typed by PCR and all were found to be of type A. The results showed that healthy broiler chickens carried several different C. perfringens clones both within a flock and even within individual birds, whereas flocks suffering from necrotic enteritis (NE) or cholangio-hepatitis carried only one or two clones.


http://www.sciencedirect.com/science/article/B6TD6-49XPTTK-1/2/8d9bd60bef5253cef966f5eb51a5b5eb

The goal of this research was to determine whether isolates of O149 porcine enterotoxigenic Escherichia coli (ETEC) recovered from recent outbreaks of severe diarrhea in weaned pigs in Ontario, Canada, had virulence attributes different from those of isolates of the same serogroup from diarrhea of pigs in the 1970s and 1980s. Polymerase chain reaction amplification was used to determine the distribution of 11 virulence-associated genes in recent (100 isolates) and old (35 isolates) Ontario O149 porcine ETEC. These tests demonstrated that 92% of the recent isolates possessed the estA gene for STa enterotoxin, whereas none of the old isolates had this gene. H antigen determination showed that all the isolates which lacked the estA gene (all 35 old isolates plus 8 recent isolates) were H43, whereas isolates which had the estA gene were H10. The astA gene for enteroaggregative heat-stable enterotoxin (EAST1) and the K88ac antigen were present in all 135 isolates. Plasmid analyses identified a cryptic 5.1 kb plasmid in 99% of recent and 60% of old isolates. Suppressive subtractive hybridization associated several types of DNA fragments with the recent O149 ETEC, namely, fragments with no homology to DNA in databases, fragments of LPS biosynthesis genes, and F plasmid DNA. We conclude that the recent outbreaks of PWD in Ontario pigs were associated primarily with a new serotype of O149 ETEC and that isolates of this serotype possessed the estA gene that was not present in old O149 ETEC isolated from pigs in Ontario.


http://www.sciencedirect.com/science/article/B6TD6-47P1T54-2/2/5d73b40094dc4100fbdbe540ed08e913

The classical swine fever (CSF) epidemic in the Netherlands in 1997-1998 lasted 14 months, during which 429 infected and 1300 at risk herds were culled, at an estimated economical cost of 2 billion US dollars. Despite the overwhelming scale of the epizootic, the CSF virus (CSFV) strain causing the outbreak has remained largely uncharacterized. The Dutch epizootic is epidemiologically linked to a small CSF outbreak in 1997, in Paderborn in Germany. E2 and partial 5' NTR sequencing has shown that the index Paderborn isolate, and several Dutch isolates taken during the 1997-1998 epizootic, are virtually identical, confirming that the Paderborn isolate triggered the Dutch outbreak, and furthermore showing that this single isolate was stable throughout the whole Dutch outbreak (the above reviewed in [C. Terpstra, A. J. de Smit, Veterinary Microbiol. 77 (2000) 3-15]). We determined the nucleotide sequence of the 5' NTR (by 5' RACE) and the complete open reading frame of the Paderborn isolate (GenBank AY072924). Our sequence was identical to previously published partial 5'NTR and E2 sequences.
for the index Paderborn 1997 and Dutch 1997 (Venhorst) isolates, confirming the identity of the virus we sequenced. Phylogenetic analysis based on the complete open reading frame showed that Paderborn is genetically very different from common European laboratory reference strains. Neutralization studies showed that Paderborn is also antigenically very different from common laboratory strains such as Alfort 187. Paderborn is the only recent European CSFV field isolate for which a complete sequence is available, and given Paderborn’s genetic and antigenic uniqueness, the Paderborn sequence may have practical use for diagnostic and vaccine antigen development.


http://www.sciencedirect.com/science/article/B6TD6-4BG3TDW-1/2/1a607c4bf6a8b0f8a4700b911b33dd38

A novel insertion element belonging to the IS110 family was identified in Mycobacterium avium subsp. paratuberculosis. The IS element, ISMpa1, is 1500 bp and has one ORF encoding a putative transposase. Three copies of ISMpa1 were identified in the M. avium subsp. paratuberculosis genome. The element had inserted into the 3' end of the highly conserved mycobacterial genes prrB and a homologue of M. tuberculosis Rv1593c, and between a putative cytochrome p450 oxygenase and a putative hydrolase. The IS element was present in all (n=11) M. avium subsp. paratuberculosis strains but not detected in most other mycobacterial species examined, including 10 M. avium subsp. avium isolates of human, avian and porcine origin. However two porcine isolates of M. avium subsp. avium and the reference strain IWGMT49 did harbour ISMpa1. These three strains belong to a previously described subgroup of M. avium subsp. avium based on IS1245 restriction fragment length polymorphism (RFLP) pattern and serovars. All of the M. avium subsp. paratuberculosis strains examined had an identical RFLP pattern when probed with sequences corresponding to the 5' end of ISMpa1, whereas a different pattern was seen in the positive M. avium subsp. avium strains. This novel IS element might be a useful tool in strain classification of M. avium subsp. avium and also for the identification of M. avium subsp. paratuberculosis when used in combination with IS900.


http://www.sciencedirect.com/science/article/B6TD6-3V7JFXV-G/2/c9cc35f6e6323241a794838131c7b35f

Faecal samples from 221, 1-30-days-old, diarrhoeic dairy calves were screened for the presence of verotoxin-producing Escherichia coli (VTEC) and eae-positive non-VTEC. Calves were grouped according to their age (1-7, 8-14, 15-21 and 22-30 days) and analyses of prevalences were done by Mantel-Haenszel [chi]2-test for trend. VTEC and eae-positive non-VTEC were detected in 20 (9.0%) and 18 (8.1%) of the diarrhoeic calves, respectively. A significant age-associated increase in the prevalence of VTEC (p=0.0001), but not in the prevalence of eae-positive non-VTEC (p=0.381), was found. Significant differences in VTEC prevalence were found between the age-group 22-30 days and in all other age-groups. 43 (5.0%) of the 861 E. coli isolates from the 221 diarrhoeic calves were VTEC, and 30 (69.8%) of these strains produced VT1 only. More than one-half of the VTEC strains (55.8%) were positive for the eae gene and all these eae-positive VTEC strains produced VT1 only. A high percentage (76.7%) of VTEC strains belonged to E. coli serogroups (O4, O26, O39, O91, O113, O128 and O145) associated with haemorrhagic colitis and haemolytic uraemic syndrome in humans. 51 (5.9%) of the E. coli strains
studied were eae-positive non-VTEC and the serogroups most prevalent among these strains were O4, O14, O26 and O123. Only four of the eae-positive strains were also espB-positive by hybridization with a probe from a human EPEC isolate and none of these strains produced VT.


http://www.sciencedirect.com/science/article/B6TD6-49H70T5C/2/9e645db4482746d0cecc6486a41164f8b

The types of the eae and espB genes of 178 attaching and effacing Escherichia coli (AEEC) strains isolated from diarrhoeic and healthy ruminants were investigated by PCR. Six types of the eae gene: [beta] (beta), [gamma]1 (gamma-1), [gamma]2 (gamma-2), [epsiv] (epsilon), [zeta] (zeta) and [iota] (iota), and three types of the espB gene: [alpha], [beta] and [gamma] were identified in the strains studied. Moreover, three strains were negative to all the types of the eae gene tested. The types [beta] and [gamma]2 in healthy cattle, [beta], [gamma]2 and [epsiv] in healthy sheep and goats, and [beta] in diarrhoeic calves, lambs and goat kids were the most frequent types of the eae gene among the strains studied. Although the eae[beta] gene was the most prevalent among AEEC from healthy and diarrhoeic ruminants, the percentages of AEEC strains with this type found in this study in diarrhoeic animals (66.7-100%) were higher than those found in healthy animals (33.3-40.6%). Thus, these data suggest that AEEC strains with the eae[beta] gene are associated with neonatal diarrhoea in ruminants. The eae[gamma]1, eae[zeta] and eae[iota] genes were found in low percentages in the strains studied (4.5, 2.8 and 7.3%, respectively). All the types of the eae gene, except the type [iota], showed a close correlation with the types of the espB gene: the eae[beta] and eae[epsiv] genes with the espB[beta] gene, the eae[gamma]2 and eae[iota] genes with the espB[alpha] gene and the eae[gamma]1 gene with the espB[gamma] gene.


http://www.sciencedirect.com/science/article/B6TD6-3XMPK3F-F/2/67949212e2a1fd3dedbb8b6f7c748932

A monoclonal antibody (mAb) that reacted specifically with a 16 kDa big liver and spleen disease virus (BLSV) protein was used to identify the protein in western immunoblots of infected liver extracts and enable partial amino acid sequence analysis of the protein. Based on this sequence, a degenerate primer was designed that was used in conjunction with random hexamers in a reverse transcriptase-POR (RT PCR), to amplify a 523 bp product from RNA extracted from homogenates of BLSV-infected livers. There was 62% nucleotide sequence identity between this sequence and the sequence of the helicase gene of human hepatitis E virus (HEV). POR primers designed from this 523 bp fragment were able to amplify a 490 bp product from livers of virus-infected chickens but not chickens from virus-free flocks.


http://www.sciencedirect.com/science/article/B6TD6-4BVRCV-
Piglet pathogenic Escherichia coli encoding Shigatoxin 2e and F18 adhesins are the etiological agents of oedema disease as well as of non-oedema disease colibacillosis. In order to reveal virulence differences among this pathogen, the presence of the pathogenicity island (PAI) E. coli type three secretion system 2 (ETT2) was examined. Using PCR and Southern blot techniques for the identification of the right, the middle, and the left region of this 29.9 kb large genetic element, the entire ETT2 was found among E. coli O138:H-, O139:H1, and O147:H6 strains originated from cases of oedema disease in Germany between 1995 and 2001 and belonging to various clonal types. In contrast, non-oedema disease E. coli isolates (e.g. O8:H19, 101:H-, O141:H4) contain deleted subtypes of ETT2. These deletions cover the translocon part of the putative ETT2-encoded type III secretion apparatus. It is suggested that the entire ETT2 is associated with a particular virulence trait of piglet oedema disease E. coli (EDEC).


http://www.sciencedirect.com/science/article/B6TD6-4BG3TDW-B/2/72f81e715a6ebf78c566621590d89cdb7

The safety and the efficacy of a modified-live (ML) canine coronavirus (CCoV) vaccine strain 257/98-3c was evaluated in 14 dogs seronegative and virus negative for CCoV. For the safety test, four dogs were inoculated, two by intramuscular and two by oronasal route, with 10 times the vaccinal dose. During the observation period (28 days) all dogs did not display any local or systemic reaction. For the efficacy test, eight dogs were vaccinated by intramuscular (four dogs--group A) or by oronasal route (four dogs--group B). Two dogs were maintained as non-vaccinated controls. In the dogs of group A, vaccinal virus was not detected in faecal samples by virus isolation (VI) and by PCR assay, while in the dogs of group B, the virus was revealed for six median days only by PCR. Twenty-eight days later, the vaccinated and control dogs were challenged with a field CCoV strain. After the challenge, the vaccinated dogs did not display clinical signs and the dogs of group A shed virus for 5.5 median days, evaluated by VI, and for 10 median days evaluated by PCR. Virus shedding was not observed, both by VI and PCR assay, in the dogs of group B. The two control dogs displayed moderate clinical signs and the virus was detected by VI for 14.5 median days starting from day 3 post-challenge (dpc 3) and by PCR assay for 23 median days starting from dpc 1.


http://www.sciencedirect.com/science/article/B6TD6-427JW5Y-6/2/cde6d22a3651ea7a004e61ba0e1b5812

Infections with atypical mycobacteria belonging to the Mycobacterium avium/intracellulare complex (MAC) can cause infection in both animals and humans. Using a standardized reagents commercial kit for random amplified polymorphic DNA (RAPD) analysis, 49 MAC strains isolated from 32 slaughter pigs and 17 humans in Sweden were identified and sorted out, yielding 6 RAPD types. By combining the results of RAPD primers 4 and 5 and the primer IS1245a, we found that pigs and humans may be infected with the same types of MAC strains, since 14 strains from humans and 8 strains from pigs were essentially identical and together, comprised RAPD type 2, the largest group of strains (44.8% of strains). With respect to grouping of strains,
serotype and RAPD type were uncorrelated, except for serotype 20 and RAPD type 6. Using standardized beads, RAPD analysis is a reproducible technique for typing MAC strains, as the indistinguishable banding patterns obtained with repeated analyses of two isolates from each strain in this study demonstrate. However, primer selection and DNA purity were crucial for differentiating closely related strains.


Mycobacterium bovis isolation on bacteriological media from suspected cases of bovine tuberculosis (TB) demands laborious and time-consuming procedures. Even polymerase chain reaction (PCR) and radiometric analyses are secondary procedures and not alternatives to bacteriological procedures. Therefore, there is a need to develop new techniques aimed at rapid M. bovis detection in diagnostic samples. The human macrophage cell line THP-1 was thus investigated in experiments of M. bovis propagation and isolation from reference lymph node suspensions. THP-1 cells were shown to support a high-titered propagation within 48 h of minute amounts of both M. bovis BCG and fully pathogenic M. bovis strain 503. A semi-nested PCR for TB-complex-specific insertion sequence IS6110 revealed M. bovis infection in THP-1 cells. The same was true of a flow cytometry (FC) assay for expression of M. bovis chaperonin 10 in infected cells. The reduced time for isolation and identification of M. bovis (48-72 h) and the consistency of the test results make the use of macrophage cell cultures attractive and cost-effective for veterinary laboratories involved in TB surveillance.


http://www.sciencedirect.com/science/article/B6TD6-44W2JX4-1/2/97839ff97d540c6ca602f8aff52a0d952

The purpose of the study reported here was to determine the prevalence of porcine reproductive and respiratory syndrome virus (PRRSV) in pigs affected with post-weaning multisystemic wasting syndrome (PMWS), a disease believed to be caused by porcine circovirus type 2 (PCV2). From May 1997 to February 2000, PMWS was diagnosed in 277 pigs (from 120 farms) submitted to the Veterinary Pathology Diagnostic Service, Veterinary School of Barcelona, Spain. In each case, the PMWS diagnosis was based on clinical history and the detection, by in situ hybridization, of nucleic acid of PCV2 in characteristic histologic lesions. Antigens for PRRSV were detected by immunohistochemistry in tissues of 66 (23.8%) of the same 277 pigs. Sera, which were available for 93 of the 277 pigs, were tested for PRRSV by a multiplex reverse transcription-polymerase chain reaction (RT-PCR). A total of 33 of these sera were RT-PCR positive, three for a North American strain(s) of PRRSV. In addition, 76 of the 93 sera were tested for antibodies to PCV2 (indirect immunoperoxidase) and PRRSV (enzyme-linked immunoassay). Antibodies for PCV2 and PRRSV were detected, respectively, in 56 (73.9%) and 43 (56.6%) of the 76 sera. Collectively, these results suggest that while infection with PRRSV may be common, it is not an essential component of PMWS.
A multiplex real-time PCR (R-PCR) assay was designed and evaluated on the ABI 7700 sequence detection system (TaqMan) to detect enterohemorrhagic Escherichia coli (EHEC) O157:H7 in pure cultures, feces, and tissues. Three sets of primers and fluorogenic probes were used for amplification and real-time detection of a 106-bp region of the eae gene encoding EHEC O157:H7-specific intimin, and 150-bp and 200-bp segments of genes stx1 and stx2 encoding Shiga toxins 1 and 2, respectively. Analysis of 67 bacterial strains demonstrated that the R-PCR assay successfully distinguished EHEC O157:H7 serotype from non-O157 serotypes and provided accurate profiling of genes encoding intimin and Shiga toxins. Bacterial strains lacking these genes were not detected with this assay. The detection range of the R-PCR assay for the three genes was linear over DNA concentrations corresponding from 10^3 to 10^8 CFU/ml of EHEC O157:H7. The R-PCR allowed construction of standard curves that facilitated quantification of EHEC O157:H7 in feces and intestinal tissues. Detection sensitivity of the R-PCR assay ranged from 10^4 to 10^8 CFU/g of feces or tissues without enrichment. Enrichment of feces in a non-selective broth for 4 and 16 h resulted in the detection of levels (from 100 to 10^3 CFU/g of feces) considered sufficient for infection in humans. The R-PCR assay for eaeO157:H7, stx1, and stx2 proved to be a rapid test for detection of EHEC O157:H7 in complex biological matrices and could also potentially be used for quantification of EHEC O157:H7 in foods or fecal samples.

Virulence factors responsible for acute diarrhea in greyhounds have not been well established. The objective of this study was to determine if a correlation exists between disease and the presence of the Escherichia coli toxin genes in non-diarrheic and diarrheic greyhound feces. DNA extracted from broth cultures was evaluated for the presence of Shiga toxin and enterotoxin genes and broth samples were evaluated for Shiga toxin and heat-labile enterotoxin. Shiga toxin (stx1 and stx2) and enterotoxin (et and estA) genes were identified in both non-diarrheic and diarrheic samples after in vitro cultured of swabs at 37 [deg]C for 16-24 h. The stx1 gene was present in 3% of non-diarrheic and 15% diarrheic samples and the stx2 gene was identified in 36 and 23%, non-diarrheic and diarrheic samples, respectively. Shiga toxin was present in 48% diarrheic and 25% of the non-diarrheic in vitro cultured samples. The elt gene was detected in vitro cultured swabs in 12% of the non-diarrheic and 7% of the diarrheic samples. Labile toxin was present in the feces of small numbers of both groups of dogs. A significant correlation existed between the presence of both stx1 genes and Shiga toxin in feces, and lack of disease in non-diarrheic (P=0.01) and presence of disease in diarrheic (P=0.024) greyhounds. Correlation between production of Shiga toxin and detection of stx1 or stx2 was significant in both the diarrheic and non-diarrheic feces (P=0.03); however, only the presence of stx1 correlated with diarrhea in both groups of samples (PE. coli in both non-diarrheic and diarrheic greyhounds indicates a zoonotic potential from dogs to humans and requires further study.
Partial nucleotide sequences were determined from the coding regions of the attachment glycoprotein (G) mRNAs of eight isolates of bovine respiratory syncytial virus (BRSV). The antigenic characteristics of 18 field and reference isolates were analyzed using the reactivity patterns of monoclonal antibodies (MAbs) directed against the human respiratory syncytial virus (HRSV) and BRSV G, fusion protein (F), nucleoprotein (N), and phosphoprotein (P), by radioimmunoprecipitation and immunofluorescence assays. The MAb reaction patterns demonstrated some random antigenic differences among the isolates, but for the most part were cross-reactive to the viral protein epitopes, especially on the F protein. Structural differences in the F and P proteins were observed among BRSV isolates; the P protein migrated at three different apparent molecular weights on PAGE gels. Antigenic and structural variation occurs among isolates, however, the structural differences in the P protein did not correlate with the antigenic differences among the F, N and P proteins. The G mRNA nucleotide sequence identities were high, ranging from 94.1 to 99.9%, and the predicted amino acid sequence identities ranged from 89.9 to 99.6%. Variance was due to substitution point mutations. The G protein ectodomains contained areas of sequence divergence flanking a highly conserved region, with four cysteine residues, which is analogous to the putative HRSV receptor binding domain. The high sequence and amino acid identities and random antigenic diversity among the isolates indicates that the BRSV isolates analyzed belong in a monophyletic group.


The 36 kDa lactate dehydrogenase (LDH) and a 29 kDa partial fragment of an ABC transporter ATP-binding protein analogue/multidrug resistance protein homologue (PR2) of Mycoplasma hyopneumoniae were tested for their potential as diagnostic antigens. Recombinant LDH was genetically engineered to contain six histidine residues at its C-terminal end, expressed in Escherichia coli and purified to a high degree using Ni2+-chelate affinity chromatography. A partial 262 amino acid segment representing the C-terminal end of the PR2 protein was cloned as a glutathione S-transferase (GST) fusion protein, expressed in E. coli and purified by urea extraction. Purified recombinant LDH-6 x His and PR2-GST were then reacted with pig sera in immunoblot assays. Our immunoblots showed that both proteins detected anti-M. hyopneumoniae antibodies in field and experimentally infected pig sera but not in any of the SPF control sera. The two proteins were specific for M. hyopneumoniae as they did not react with sera of pigs infected with the closely related Mycoplasma flocculare and Mycoplasma hyorhinis which are frequently isolated in pigs but are not of particular concern.

We have constructed a physical map of the Mycoplasma agalactiae strain PG2 chromosome analyzing it by pulsed field gel electrophoresis in a contour-clamped homogeneous electric-field system. We mapped 33 cleavage sites generated with SmaI, XhoI, SalI, EclXI and BsiWI restriction endonucleases using double digestions, one- and two-dimensional pulsed electrophoresis, cross-hybridization and linking clones. We have also mapped the loci of some genes by Southern hybridization.

http://www.sciencedirect.com/science/article/B6TD6-4FR8PMD-2/2/7988fac2bb6a2e7db0b5feafad9ad850

Colibacillosis appears to be of increasing importance in layer flocks. The aim of this study was to determine characteristics of avian pathogenic Escherichia coli associated with the occurrence of colibacillosis outbreaks at flock level. Forty E. coli strains originating from layers from healthy flocks (‘control isolates’), consisting of 25 caecal and 15 extra-intestinal isolates, were compared with 40 strains isolated from layers originating from colibacillosis-affected flocks (‘outbreak isolates’), consisting of 20 caecal and 20 extra-intestinal isolates. The examined characteristics were adhesins, invasivity in T84 cell culture, serum resistance, iron uptake, colicin production, and toxigenicity. The following traits were significantly more often detected in the outbreak isolates than in the control isolates: tsh, iss, iucA, iutA, irp2, fyuA, iroC, cvaC, colicin and colicin V production. A comparison of the extra-intestinal outbreak isolates and the caecal control isolates yielded the same results as when the caecal isolates, extra-intestinal isolates and total number of isolates of the outbreak and the control group were compared. When comparing the caecal and extra-intestinal isolates within the control and within the outbreak group, no significant differences were detected. The O78 and O2 groups showed significant differences with other O-types and NT strains for prevalence of most of the same characteristics. The combination of type 1 fimbriae, tsh, serum resistance, iss, traT, iucA, fyuA, iroC and colicin or colicin V production was significantly more often present in extra-intestinal outbreak isolates than in extra-intestinal control isolates. Only the combination of serum resistance, fyuA and colicin production was present in all outbreak isolates, with a significantly lower prevalence in the control isolates. None of the characteristics or combinations examined were exclusive to the outbreak isolates.

http://www.sciencedirect.com/science/article/B6TD6-4B2CNMM-2/2/4d8422faaba1012e6545456d5280053c

The course of enzootic pneumonia, caused by Mycoplasma hyopneumoniae, is strongly influenced by management and housing conditions. Other factors, including differences in virulence between M. hyopneumoniae strains, may also be involved. The aim of this study was to evaluate the virulence of six M. hyopneumoniae field isolates and link it to genetic differences as determined by randomly amplified polymorphic DNA (RAPD) analysis. Ninety, conventional M. hyopneumoniae-free piglets were inoculated intratracheally with the field isolates, a virulent reference strain or sterile culture medium. Animals were examined daily for the presence of disease signs and a respiratory disease score (RDS) was assessed per pig. Twenty-eight days post infection, pigs were euthanized, blood sampled and a lung lesion score was given. Lung samples were processed for histopathology, immunofluorescence testing for M. hyopneumoniae and isolation of M. hyopneumoniae. RAPD analysis was performed on all M. hyopneumoniae strains. Significant differences between isolates were found for the RDS, lung lesion score,
histopathology, immunofluorescence and serology. Based on the results of the different parameters, isolates were divided into three “virulence” groups: low, moderately and highly virulent strains. Typically, a 5000 bp RAPD fragment was associated with the highly and moderately virulent strains whereas it was absent in low virulent strains. It was concluded that high variation in virulence exists between M. hyopneumoniae strains isolated from different swine herds. Further studies are required to determine whether the 5000 bp fragment obtained in the RAPD analysis can be used as a virulence marker.


http://www.sciencedirect.com/science/article/B6TD6-476TTW8-H/2/b2ef78ab562c02155fdd7c170ee9a1c7

This paper reports a naturally occurring case of meningoencephalitis associated with Listeria innocua in a Polled-Dorset ewe. The ewe was one of a housed group of twenty-five, fed ad lib. on wrapped baled silage. L. innocua was isolated after one week from cold enrichment culture of brain and pituitary tissue. Its identity was confirmed by conventional biochemical tests, API Listeria (BioMerieux UK Ltd), the absence of hly and prfA genes using PCR assay and sequencing two variable regions of 16S rDNA. Histological examination demonstrated lesions of vasculitis and perivascular cuffing in the midbrain which were consistent with listeriosis although limited in distribution and severity.


http://www.sciencedirect.com/science/article/B6TD6-3YS8YC7-P/2/ca1c465321b4f8086049395c9f229328

A polymerase chain reaction (PCR) test was developed to detect Mycobacterium bovis in tissues. The test was based on amplification of a 248 bp segment of the insertion sequence, IS1081, present in six copies in strains of M. bovis and other members of the tuberculosis complex. The procedure involved digestion with proteinase K, lysis with sodium dodecyl sulphate, and extraction with hexadecyl tetramethyl ammonium bromide and phenol:chloroform:iso-amyl alcohol. When agarose gel electrophoresis was used for detection, the method was able to detect 1 fg of pure DNA, or 0.2 genome equivalents. It could also detect as few as 10 organisms from pure cultures and between 200-500 organisms from tissues spiked with cultured organisms. Detection by hybridization was only marginally more sensitive. The method was tested on 110 selected tissues recovered post mortem from a variety of animals. Fifty three of 58 samples diagnosed as M. bovis culture positive, including all samples containing microscopically visible acid-fast bacilli, were positive on duplicate testing by PCR. Five of 52 culture negative samples were also positive by PCR including three which contained large numbers of acid-fast organisms. Ten of the culture negative samples came from animals in a herd known to be free of bovine tuberculosis and all these were negative by PCR.

We developed a PCR assay for the rapid and sensitive detection of virulent Streptococcus suis type 2 and highly virulent S. suis type 1 in tonsillar specimens from pigs. The PCR primers were based on the sequence of the gene encoding the EF-protein of virulent S. suis type 2 strains (MRP+EF+) and highly virulent S. suis type 1 strains (MRP+EF+) and of the EF* protein of weakly virulent S. suis type 2 strains (MRP+EF*). The latter strains give rise to larger PCR products than the virulent strains of S. suis type 1 and 2. A positive control template was included in the assay to identify false negative results. The PCR was evaluated using tonsillar specimens from herds known (or suspected) to be infected and herds without an S. suis history. The results obtained with the PCR assay were compared with the results obtained with a newly developed bacteriological examination. In this bacteriological examination we were able to identify the EF-positive strains directly in the tonsillar specimens. From the 99 tonsils examined, 48 were positive in the PCR and 51 negative. All specimens from which EF-positive S. suis strains were isolated were also positive in the PCR assay. Three samples were positive in the PCR, but negative by bacteriological examination. The results demonstrated that the PCR is a highly specific and sensitive diagnostic tool for the detection of pigs carrying virulent strains of S. suis type 2 and highly virulent strains of type 1. Application of the assay may contribute to the control of S. suis infections.


Oligonucleotide primers were designed for the specific polymerase chain reaction (PCR) amplification of the enterotoxins STIa and LTI and of the verocytotoxins VT1 and VT2. All of 184 E. Coli isolates from cases of diarrhoea from pigs, cattle and sheep gave identical toxin profiles by PCR and gene probe. Differentiation between VT2 and VT2v was achieved using two oligonucleotide primers pairs in PCR and showed that all of 34 VT2+ porcine isolates, of which 23 were 0138:K1, harboured VT2v whereas 20 VT2+ bovine and ovine isolates harboured VT2. No isolate harboured both VT2 polymorphs. Simplified methods for sample preparation for PCR were examined and showed that PCR was not inhibited by direct addition of broth culture to the reaction mixture.

acid residue differences observed. The deoxynucleotide sequences showed genetic drift with alternative bases in the third position of codons. The PCR product derived by amplification of flaB from L. grippotyphosa was cloned into the expression vector pGEX-2T and a recombinant FlaB fusion protein made. As predicted from the deduced amino acid sequences, the recombinant FlaB cross-reacted with heterologous antiserum derived from a rabbit infected with L. hardjo-bovis.


http://www.sciencedirect.com/science/article/B6TD6-405KDD3-5/2/b5873a86dd4cebeefa2aea80d2d8f87

In late summer through early winter of 1998, there were several outbreaks of respiratory disease in the swine herds of North Carolina, Texas, Minnesota and Iowa. Four viral isolates from outbreaks in different states were analyzed, both antigenically and genetically. All of the isolates were identified as H3N2 influenza viruses with antigenic profiles similar to those of recent human H3 strains. Genotyping and phylogenetic analysis demonstrated that the four swine viruses had emerged through two different pathways. The North Carolina isolate is the product of genetic reassortment between human and swine influenza viruses, while the others arose from reassortment of human, swine and avian viral genes. The hemagglutinin genes of the four isolates were all derived from the human H3N2 virus circulating in 1995. It remains to be determined if either of these recently emerged viruses will become established in the pigs in North America and whether they will become an economic burden.

Zhou, W., R. F. Cook, et al. (2002). "Multiple RNA splicing and the presence of cryptic RNA splice donor and acceptor sites may contribute to low expression levels and poor immunogenicity of potential DNA vaccines containing the env gene of equine infectious anemia virus (EIAV)." Veterinary Microbiology 88(2): 127.

http://www.sciencedirect.com/science/article/B6TD6-46BMTXT-2/2/d62052c6b55bfc208576930e9a6413dfa

The env gene is an excellent candidate for inclusion in any DNA-based vaccine approach against equine infectious anemia virus (EIAV). Unfortunately, this gene is subjected to mutational pressure in E. coli resulting in the introduction of stop codons at the 5' terminus unless it is molecularly cloned using very-low-copy-number plasmid vectors. To overcome this problem, a mammalian expression vector was constructed based on the low-copy-number pLG338-30 plasmid. This permitted the production of full-length EIAV env gene clones (plcnCMVenv) from which low-level expression of the viral surface unit glycoprotein (gp90) was detected following transfection into COS-1 cells. Although this suggested the nuclear export of complete env mRNA moieties at least two additional polypeptides of 29 and 20 kDa (probably Rev) were produced by alternative splicing events as demonstrated by the fact that their synthesis was prevented by mutational inactivation of EIAV env splice donor 3 (SD3) site. The plcnCMVenv did not stimulate immune responses in mice or in horses, whereas an env construct containing an inactivated SD3 site (plcnCMV[Delta]SD3) did induce weak humoral responses against gp90 in mice. This poor immunogenicity in vivo was probably not related to the inherent antigenicity of the proteins encoded by these constructs but to some fundamental properties of EIAV env gene expression. Attempts to modify one of these properties by mutational inactivation of known viral RNA splice sites resulted in activation of previously unidentified cryptic SD and splice acceptor sites.