CONTROL ID: 2021079

TITLE: Isolation of a Novel *Campylobacter* Species from Bovine Genital Sample

ABSTRACT BODY:

Narrative: *Campylobacter fetus* comprises two subspecies, *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis*. *C. fetus* subsp. *venerealis* causes bovine venereal campylobacteriosis (BVC), a sexually transmitted disease of cattle that if not properly controlled leads to severe economic loss particularly to industries with focus on breeding programs. Therefore, accurate identification of the organism is essential. *C. fetus* subsp. *venerealis* can be differentiated from closely related *Campylobacter* spp. by their growth behavior in 1% glycine and susceptibility pattern on cephalothin followed by confirmation with molecular method. At the Wisconsin Veterinary Diagnostic Laboratory we use a real-time polymerase chain reaction (rt-PCR) assay that simultaneously targets a gene that is present in *C. fetus* and a gene that is reportedly unique to *C. fetus* subsp. *venerealis*. Recently, a slow growing, gram-negative curved bacterium was recovered from bovine vaginal wash and swab. The organism exhibited *C. fetus* subsp. *venerealis* characteristics by biochemical and susceptibility patterns and was negative by PCR for *Campylobacter fetus* but positive for *C. fetus* subsp. *venerealis*. Comparative 16S sequence based identification analysis indicated that the isolate belongs to the genus *Campylobacter*. Cluster analysis derived from the 16S rDNA sequences revealed that the isolate is more closely related to other campylobacters than to *C. fetus* subsp. *venerealis*. Genotyping by Pulsed Field Gel electrophoresis (PFGE) did not produce meaningful results. Further, the matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) and cellular fatty acid methyl ester analysis yielded no identification. These findings suggest that the isolate may be novel and deserves further study as potential veterinary pathogen.

CURRENT CATEGORY/DISCIPLINE: Bacteriology/Mycology

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AWARDS:

Trainee Letter:

CONTROL ID: 2020773

TITLE: Isolation and Identification of *Helcococcus ovis* from Bovine Joint Fluid

ABSTRACT BODY:

Narrative: Lung tissue and joint fluid were submitted to the University of Missouri College of Veterinary Medicine Veterinary Medical Diagnostic Laboratory for Bacteriology and Molecular Diagnostic examination. The samples were obtained from a Holstein calf with clinical signs of possible pneumonia and swollen joints. As per standard operational procedure the submitted samples were cultured aerobically on Blood and MacConkey agars as well as under microaerophilic conditions (5% CO2, 95% air) on Chocolate and Eugon Chocolate Agars. Both submitted samples were found to contain mixed microbial growth. The lung contained light growth of isolates subsequently identified as *Corynebacterium*, *Staphylococcus* and *Streptococcus* species. The joint fluid contained light growth of a hemolytic *Bacillus* on aerobic culture and heavy growth of minute grey colonies on Chocolate and Eugon Chocolate agars cultured in the presence of CO2. This latter isolate was found to be KOH and catalase negative and appeared microscopically as paired Gram-positive cocci resembling *Micrococcus*. Automated bacterial identification systems (Thermo Fisher Scientific Sensititre AP90 Gram-positive Identification system) failed to identify the isolate even to the level of genus. Ribosomal RNA (16S) sequencing was employed as a diagnostic tool to identify this unusual microbe. Results from sequencing showed one nucleotide difference out of 1423 base pairs between this isolate and the *Helcococcus ovis* type strain s840-96-2. *Helcococcus ovis*, a facultative anaerobic Gram positive catalase negative cocci

was first recovered together with *Trueperella pyogenes* from multiple tissues during post mortem examination of two adult sheep in Scotland. Like that report we have demonstrated an aerobic growth dependency on *Staphylococcus aureus* and are unaware of the clinical significance of this isolate in the joint fluid of the bovine. Independent molecular evaluation of the submitted samples demonstrated that the lung was PCR positive for *Mycoplasma*, but not *M. bovis*; the joint fluid was *Mycoplasma* negative by PCR. Microbiological and molecular testing is still ongoing.

CURRENT CATEGORY/DISCIPLINE: Bacteriology/Mycology

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AWARDS:

Trainee Letter:

CONTROL ID: 2019343

TITLE: A Taqman Multiplex Real-Time PCR for Rapid Detection of Viral and Bacterial Pathogens Associated with Bovine Respiratory Disease Complex

ABSTRACT BODY:

Narrative: Bovine Respiratory Disease Complex (BRDC) is the most significant infectious disease in cattle. The disease is multifactorial, with either stress or reduced immunity allowing primary and/or secondary infections by viral and bacterial pathogens to occur. *Bovine viral diarrhea virus* (BVDV), *Bovine respiratory syncytial virus* (BRSV), *Bovine coronavirus* (BCoV) and *Bovine herpesvirus* type 1 (BHV-1/IBR), *Mannheimia haemolytica* (Mh), *Pasteurella multocida* (Pm), *Histophilus somni* (Hs), *Bibersteinia trehalosi* (Bt) and *Mycoplasma bovis* (Mb) are 9 pathogens that are commonly associated with BRDC. A Taqman multiplex real-time PCR assay consisting of three triplex reactions was developed and validated for simultaneous detection of these 9 pathogens involved in BRDC. The complete panel includes a triplex reaction for detection of the BRD viral RNA pathogens (BVD, BRSV and BCoV), a triplex reaction for DNA pathogens (*M. bovis*, BHV1/IBR and *Pasteurella multocida*) and the third triplex assay for the detection of the BRD bacterial pathogens: *M. haemolytica*, *H. somni* and *B. trehalosi*. Each individual singleplex reaction was optimized separately and subsequently multiplexed, with PCR amplification efficiencies of 92.1-105.4% and correlation coefficients of 0.99-0.999. Assay specificity was assessed by testing non-target and closely related species and there was no cross-reactivity observed. Comparison of detection limit and reaction efficiency between singleplex and multiplex assays indicated that multiplexing does not inhibit the detection sensitivity of the assay. Testing on 70 clinical samples using the assay showed good sensitivity and specificity. This study provides the basis for further evaluating the assay's diagnostic performance in clinical service for rapid detection of viral and bacterial pathogens associated with Bovine Respiratory Disease Complex (BRDC).

CURRENT CATEGORY/DISCIPLINE: Bacteriology/Mycology | Virology

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AWARDS:

Trainee Letter:

CONTROL ID: 2022871

TITLE: Development and Evaluation of a Real-Time PCR Assay for Detection of *Salmonella* Heidelberg from Clinical and Environmental Samples

ABSTRACT BODY:

Narrative: *Salmonella* Heidelberg represents an emerging health concern both for veterinary patients and humans due to zoonotic transmission. Recent food-borne outbreaks related to this serotype have also demonstrated the potential hazard it presents to the safety of our human food supply. While there are currently no demands for regulatory testing related to S. Heidelberg, management and testing requirements may be forthcoming in an attempt to reduce its impact from livestock and poultry food product sources. Twenty-one S. Heidelberg isolates along with 21 non-S. Heidelberg isolates recovered from environmental and clinical submissions to the California Animal Health and Food Safety Lab System (CAHFS) were submitted for whole-genome sequencing and analysis was performed to determine if a suitable gene target could be identified for PCR detection. After preliminary evaluation in silico, and a putative Type II restriction enzyme sequence was selected for further evaluation. A real-time PCR assay was developed that identified a 62 base-pair target in this region conserved in S. Heidelberg but not present in other serotypes. PCR testing on a panel of 26 different S. Heidelberg isolates demonstrated 100% detection utilizing this target. When tested against 22 non-Heidelberg *Salmonella* sp. organisms, 21 of the strains generated negative results to this target. The single discrepant result yielded a PCR product from a S. Newport isolate with a Ct of 36.8 in which a large concentration of DNA (403 ng/µl) was tested; dilution of this sample to a concentration closer to that obtained for other isolates tested in this assay (140 ng/µl) did not result in a detectable PCR product on repeat testing. Consistent detection was obtained with concentrations of 1.5 X 103 cfu/ml in broth samples without enrichment. No interference was seen when samples were co-inoculated with S. Heidleberg and non-Heidelberg *Salmonella* serotypes. Side by side comparisons between this real-time PCR assay and traditional culture with serotyping performed on tetrathionate broths from twenty field samples incubated for 18-24 hours at 42 °C yielded 100% agreement between the two methods. This assay may prove to be useful for sensitive and specific detection of S. Heidelberg in clinical and environmental samples.

CURRENT CATEGORY/DISCIPLINE: Bacteriology/Mycology | Epidemiology

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AWARDS:

Trainee Letter:

CONTROL ID: 2022550

TITLE: Prevalence, isolation and characterization of *E. coli* O104 in cattle feces

ABSTRACT BODY:

Narrative: The hybrid serotype of *E. coli* O104:H4, reported in 2011 outbreak in Germany, possessed characteristics of two pathotypes, Shiga toxin-producing and enteroaggregative *E. coli*. Although O104:H4 has not been detected in cattle, other serotypes (O104:H21, O104:H2, O104:H7, O104:H12) have been detected in cattle and other animals. The objectives of our study were to determine the prevalence of *E. coli* O104 in feedlot cattle feces, isolate and characterize the strains. A total of 757 rectal content samples from cattle, representing 29 different feedyards, were collected at a slaughter plant in July, 2013. Fecal samples were enriched in *E. coli* broth for 6 h at 40 C and then subjected to PCR- and culture-based methods

of detection. DNA extracted from pre and post enriched fecal samples were tested by a multiplex PCR to detect serogroup O104 and associated virulence genes (*terD*, *ehxA*, *stx*1, *stx*2, *bfpA*, *aggA*, *eae* and *flicH4*) of the hybrid pathotype. Culture-based method of detection involved immunomagnetic separation with O104 beads, plating on selective chromogenic medium, followed by serogroup confirmation by PCR. Isolates of O104 were tested by PCR assays to determine capsular antigen of O8/O9 serogroups, virulence genes and flagellar types. Of the 757 samples, 38(5%) and 349 (46%) samples were positive before and after enrichment respectively for O104 serogroup-specific gene. A total of 143 O104 isolates were obtained and 92 of them were positive for O8/O9 capsular antigen genes. Of the 51 O104 isolates, only 16 (31.4%) carried *stx*1, none of them carried *eae*. Subtyping of *stx*1 was performed based on amino acid sequences and all 16 isolates carried *stx*1c. *In silico* restriction fragment length polymorphism (RFLP) was performed to compare with the subtyping based on amino acid sequences. *In silico* RFLP was also compared with conventional PCR-RFLP. The results from PCR/RFLP and *in silico* RFLP were in agreement with those obtained by subtyping based on amino acid sequences. The O104 isolates harbored diverse flagellar (H) antigens with 36 isolates containing H7, 4 H2, 1 each of H21 and H1. Pulsed-field gel electrophoresis (PFGE) was performed to assess the genetic relatedness of the *stx*-positive O104 isolates. The 16 *stx*1-positive isolates formed three PFGE clusters and the 13 isolates from one feedyard were of the same PFGE subtype (100% similarity). Cattle shed serogroup O104 in feces, but only a few strains (11.2 %) carried *stx*1 gene and none of the isolated strains carried genes characteristic of the hybrid pathotype.

CURRENT CATEGORY/DISCIPLINE: Epidemiology

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AWARDS: Graduate Student Oral Presentation

Trainee Letter: trainee letter-2014.pdf

CONTROL ID: 2021612

TITLE: An Improved Primer Set for the Genotyping of *Clostridium perfringens*

ABSTRACT BODY:

Narrative: *Clostridium perfringens* is a widely distributed Gram-positive bacterium responsible for a wide range of diseases in humans and animals. *C. perfringens* strains are categorized into five toxinotypes (A-E) according to their complement of four major toxin genes. There is an important variation within the cpa gene, which encodes the alpha toxin present in all toxinotypes of *C. perfringens*. In addition, the gene encoding Beta2 toxin is present as two alleles with ~70% sequence homology and substantial sequence variation in each allele. Collectively, this presents a diagnostic challenge for accurately genotyping isolates for the cpa and cpb2 genes. This study focused on strain variation between isolates from calves and the effect of this variation on genotyping accuracy. New primer sets for amplifying cpa and cpb2 were designed based on all known sequence variants of the cpa and cpb2 genes. The new primer set identified isolates carrying cpb2 and cpa in samples that did not show bands corresponding to these genes using previously published and widely used primer sets. This primer set represents an important improvement in the diagnostic genotyping of *C. perfringens* field isolates.

CURRENT CATEGORY/DISCIPLINE: Bacteriology/Mycology | Pathology

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AWARDS: Graduate Student Poster Presentation|Graduate Student Oral Presentation

Trainee Letter:

CONTROL ID: 2021335

TITLE: Survey of Respiratory Pathology in Wild Urban Rats (*Rattus norvegicus* and *Rattus rattus*)

ABSTRACT BODY:
Narrative: Norway (*Rattus norvegicus*) and black rats (*R. rattus*) are common commensal pests that live in association with human habitats and carry a number of zoonotic pathogens, yet we know little about wild rat ecology, including their natural diseases. We describe the gross and histological lesions of the respiratory tract in a sample of 711 wild urban rats. Over a 1-year period, rats were trapped from an inner city neighborhood in Vancouver, Canada and autopsied. A subset was examined for 19 distinct categories of histological lesions in the respiratory tract. Samples were tested, using serology and PCR, for known rat respiratory pathogens. Grossly-evident lesions were rare (8/711; 1%). Upper respiratory tract (URT) inflammation (rhinitis, submucosal and periglandular lymphoplasmacytic tracheitis, and/or tracheal intraluminal necrotic debris) was present in 93/107 (87%) rats and was associated with Cilia Associated Respiratory Bacillus (CARB) and *Mycoplasma pulmonis* ($P \leq .05$), and heavier rats (OR = 1.09, 95% CI = 1.05 – 1.14 per 10 g). Lower respiratory tract (LRT) inflammation (peribronchiolar and/or perivascular lymphoplasmacytic cuffing) was present in 152/199 rats (76%) and inflammation was associated with CARB and *M. pulmonis* ($P \leq .02$), and heavier rats (OR = 1.20, 95% CI = 1.14 – 1.27 per 10 g). *Pneumocystis carinii* was detected in 48/102 (47%) rats using PCR, but was not significantly associated with lesions. This is the first detailed description of pathology in the respiratory system of wild rats and demonstrates that respiratory disease is common. Although the impact of these lesions on individual and population health remains to be investigated, respiratory disease may be an important contributor to wild rat morbidity and mortality.

CURRENT CATEGORY/DISCIPLINE: Pathology | Bacteriology/Mycology

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AWARDS: Trainee Travel Award|Graduate Student Poster Presentation|Graduate Student Oral Presentation

Trainee Letter: AAVLD Trainee Travel Awards Applicant Letter_JR.pdf

CONTROL ID: 2022690

TITLE: Minimal Inhibitory Concentration Antimicrobial Susceptibilities of Coagulase Positive *Staphylococcus sp.* Associated with Canine Pyoderma (2011-2013)

ABSTRACT BODY:
Narrative: Skin swabs or skin biopsies from dogs with pyoderma were submitted to the University of Illinois Veterinary Diagnostic Laboratory for bacterial culture and susceptibility testing as part of a study investigating potential for exchange of antimicrobial resistance and/or virulence factors between *Staphylococcus species* during mixed infections. Dominant among the isolates recovered were coagulase positive *Staphylococcus species*, predominantly *Staphylococcus pseudintermedius* (N=327), *Staphylococcus schleiferi* (N=38) and *Staphylococcus aureus* (N=17). Isolates were identified by Gram stain, catalase, coagulase, ONPG broth, and/or Sensititre™ GPID or Biolog™ GP2 systems as needed. Only 12 dogs had significant co-infections with multiple *Staphylococcus species*, *S. pseudintermedius* with either *S. aureus* or *S. schleiferi*. Susceptibilities were performed using Sensititre™ Companion Animal 1F or 2F MIC panels, following Clinical Laboratory Standards Institute M31-A3 and Vet01-A4/S2 guidelines and interpretations. Penicillin resistance was common among *S. pseudintermedius* isolates (MIC50=1.0 µg/ml, MIC90=16.0 µg/ml) while *S. schleiferi* were more susceptible (MIC50≤ 0.06 µg/ml, MIC90=1.0 µg/ml). A similar trend was observed for ampicillin with a *S. schleiferi* MIC90 = 1.0 µg/ml and MIC90 ≥ 4.0 µg/ml for both *S. pseudintermedius* and *S. schleiferi*. Based upon oxacillin breakpoints of 4.0 µg/ml for *S. aureus* and 0.5 µg/ml for *S. pseudintermedius* and *S. schleiferi*, 35.3%, 30.4% and 34.2% of these isolates exhibited methicillin resistance, respectively. While the MIC50 values were all 0.25 µg/ml, MIC90 values were 8.0, 4.0 and 2.0, respectively. There was good correlation between the oxacillin breakpoints and the presence of the *mecA* gene detected by PCR. Fluoroquinolone resistance was more common among *S. schleiferi* isolates (MIC50=1.0 µg/ml , MIC90=2.0 µg/ml), and both *S. aureus* and *S. pseudintermedius* had MIC50 values of ≤0.25 µg/ml but MIC90 values of ≥ 4.0 µg/ml for both enrofloxacin and marbofloxacin. The prevalence of methicillin resistance among coagulase positive Staphylococci is much higher for dogs with pyoderma (~30%) than the prevalence reported for healthy dogs (~6% for methicillin resistance) possibly as a result of selection pressure associated with antibiotic treatment.

CURRENT CATEGORY/DISCIPLINE: Bacteriology/Mycology

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AWARDS:

Trainee Letter:

CONTROL ID: 2022625

TITLE: Virulence Factors Genes (siet, lukI and lukF) in *Staphylococcus* Species Isolated from Canine Clinical Specimens

ABSTRACT BODY:
Narrative: The epidemiology and study of virulence factors in methicillin-resistance (MR) *S. pseudintermedius* and *S. schleiferi* subsp. *coagulans* isolated from dogs is sparse. The aim of this study was to investigated the presence of the genes siet, lukI and lukF, which encode for important virulence factors in *S. schleiferi* subsp. *coagulans*, *S. pseudintermedius* and *S. aureus* isolated from canine clinical specimens. In addition, related epidemiological information related with previous antimicrobial therapy, breed, gender, age and prevalence of methicillin-resistant isolates by year was analyzed. A total of 577 coagulase positive *Staphylococcus* species were collected from canine clinical specimens, and epidemiological information was collected from the clinical history submitted to the lab. The incidence of the genes siet, lukI and lukF were determined by PCR. A significant difference ($p \leq 0.05$) indicated that isolates from dogs with a history of antimicrobial therapy have a higher risk of being MR positive, than from dogs without therapy. Within the *S. aureus* group, the panton-

valentine leukocidin genes were tested in the MRSA group with four testing positive, 12.9% (4/31). LUK-I and siet genes were found mainly in *S. pseudintermedius*, but also in *S. aureus* and *S. schleiferi* subsp. *coagulans*. An important finding in this study was the discovery of the genes siet and lukI in *S. aureus* and *S. schleiferi* subsp.*coagulans*. Collectively, these findings support the need for further epidemiological and clinical studies concerning virulence factors in *Staphylococcus* species of canine origin, which may be a potential hazard for public health.

CURRENT CATEGORY/DISCIPLINE: Bacteriology/Mycology | Epidemiology

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AWARDS:

Trainee Letter:

CONTROL ID: 2010678

TITLE: Fatalities Caused By *Streptococcus dysgalactiae* subsp. *equisimilis* in Horses

ABSTRACT BODY:

Narrative: *Streptococcus dysgalactiae* subsp. *equisimilis* (*S. equisimilis*) causes severe clinical syndromes in humans with high mortality rates including arthritis, pleuropulmonary infections, peritonitis, intra-abdominal and epidural abscesses, meningitis, endocarditis, puerperal septicemia, neonatal infections, necrotizing fasciitis, myositis, and streptococcal toxic-like syndrome. *S. equisimilis* has been reported in horses from aborted placenta, less frequently from abscessed lymph nodes, and the upper respiratory system infections in horses. However, fatalities caused by *S. equisimilis* in horses are poorly described and, comprehensive and exhaustive studies are limited. The goal of this study is to describe fatalities of horses caused by *S. equisimilis* and examine the genotypes of equine isolates of *S. equisimilis* by using multilocus sequence analysis, and M virulence protein gene (emm) typing combined with phenotypic characterization.
105 equine necropsies where *S. equisimilis* were isolated were retrospectively analyzed. The necropsies were performed at the University of Kentucky Veterinary Diagnostic Laboratory between January 1, 2010 and December 31, 2013. In 22 cases, *S. equisimilis* was reported as only causative microorganism leading to death. In 15 of these cases, *S. equisimilis* caused abortion due to placentitis with or without fetal septicemia. *S. equisimilis* also caused osteoarthritis/ osteomyelitis/arthritis (5 cases), and septicemia (2 cases). In 29 other cases, *S. equisimilis* was diagnosed as contributing or associated agent to the fatality (or euthanasia) causing placentitis/abortion (9 cases), pleuritis (4 cases), pneumonia (4 cases), septicemia (4 cases), dermatitis/ cellulitis/ myelitis (3 cases), osteomyelitis/arthritis (2 cases), cystitis (1 case), omphalitis (1 case), and mesenteric abscess (1 case). Other contributing microorganisms identified in these cases were *S. aureus*, *S.equi* subsp. *zooepidemicus*, *P. caballi*, *Neorickettsia risticii*, *R. equi*, *A. equi* subsp. *equi*, *K. pneumonia*, *E. coli*, *Enterococcus* spp and *Streptococcus* spp. In 54 necropsy cases, *S. equisimilis* was recovered from tissues but not associated with any disease/fatality.
12 *S. equisimilis* isolates representing these groups were confirmed to be *S. equisimilis* by PCR using streptokinase gene specific primers. In addition, multilocus sequence types (MLSTs) and sequence types of the emm gene were obtained and compared to human disease case *S. equisimilis* isolates. These preliminary results shown that these *S. equisimilis* isolates recovered from equine necropsy cases have new MLSTs and emm types suggestive of a genetic lineage distinct from human isolate clonal complexes described to date.

CURRENT CATEGORY/DISCIPLINE: Bacteriology/Mycology

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AWARDS:

CONTROL ID: 2010731

TITLE: A Novel *Avibacterium* sp. Causes Mortality in Laying Hens

ABSTRACT BODY:

Narrative: In January 2014, a disease outbreak characterized by elevated mortality and reduced egg production occurred in a commercial in-line high-rise laying hen operation. White leghorn chickens in 3 of 9 houses on this farm were affected. Each house had an average capacity of 111,000 birds and affected flocks ranged in age from 39 to 48 weeks. The flock manager reported mildly swollen heads and conjunctivitis in a few hens housed in each of these three flocks. Macroscopic lesions consisted primarily of hepatosplenomegaly, air sacculitis, and pneumonia. Abundant caseous exudate was present in anterior thoracic air sacs and lungs were consolidated with exudate in bronchi. Livers, lungs, air sacs, and spleens were collected using aseptic technique and pooled. All samples were cultured using routine procedures on blood agar plates under aerobic and anaerobic conditions. *Avibacterium* sp. was isolated from lungs and air sacs and *E. coli* was isolated from livers. Biochemical tests suggested that *Avibacterium* isolated from these chickens was not *Avibacterium paragallinarum*, the etiologic agent associated with infectious coryza. To identify the species of *Avibacterium*, the 16S ribosomal gene of selected isolates was amplified by PCR and sequenced. The 16S rRNA phylogenetic tree of veterinary *Pasteurellaceae* species confirmed that this isolate is not *A. paragallinarum* and is different from other *Avibacterium* sp. previously isolated from chickens in the United States.

CURRENT CATEGORY/DISCIPLINE: Bacteriology/Mycology

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AWARDS:

Trainee Letter:

CONTROL ID: 2021346

TITLE: Development of a Multiplex PCR Assay for the Identification and Speciation of Pathogenic Vibrionaceae

ABSTRACT BODY:

Narrative: There are currently several emerging pathogens within the family Vibrionaceae that are causative agents of disease in both humans and fish. Identification and speciation of these organisms using classical culture and biochemical methods yield results that are frequently inconsistent, necessitating diagnostic techniques that are accurate, rapid, and cost effective for proper Vibrionaceae identification. Multiplex PCR has proven useful for *Vibrio* spp. in previous studies, although these tests have typically been limited to human pathogens such as *Vibrio cholerae*. We have designed a multiplex PCR assay for the detection of six fish and human pathogens within the Vibrionaceae family: *Vibrio alginolyticus, Vibrio (Listonella)*

anguillarum, *Vibrio ordalii*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, and *Photobacterium damselae* subsp. *damselae*. Each of these species was correctly and consistently identified when tested against their corresponding American Type Culture Collection (ATCC) strains and known clinical isolates, and no reaction was observed when tested against a variety of non-*Vibrio* isolates. Fifty-five presumptive *Vibrio* spp. clinical isolates were additionally tested by multiplex PCR, MALDI-TOF mass spectrometry, and sequencing of multiple gene targets. There was a high degree of concordance between mass spectrometry and multiplex PCR results, though PCR showed a greater ability to differentiate between *V. anguillarum* and *V. ordalii* isolates. The PCR assay demonstrated high accuracy compared to other available techniques and is also highly modular, as it can be successfully run as triplexes or a single hexaplex reaction and retains the potential of having additional primer sets incorporated into it for the detection of other *Vibrio* spp. This multiplex PCR assay represents a useful diagnostic tool for the proper identification of multiple Vibrionaceae species that will be beneficial for aquaculture and in both veterinary and human medicine.

CURRENT CATEGORY/DISCIPLINE: Bacteriology/Mycology

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AWARDS:

Trainee Letter: Seth Nydam Trainee Role.pdf

CONTROL ID: 2021359

TITLE: Use of Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) for the Speciation of Pathogenic *Vibrio* in Fish

ABSTRACT BODY:

Narrative: Many *Vibrio* spp. cause disease resulting in significant morbidity and mortality in farmed fish and significant economic losses to fish producers around the world. Speciation of *Vibrio* isolates is time consuming, difficult, and costly due to species diversity and inconsistency of phenotypic and biochemical traits within species. Therefore, improved methods of *Vibrio* speciation would aid identification accuracy and improve disease diagnosis. MALDI-TOF MS has been shown to be a rapid and cost effective method for speciation of numerous bacteria, but its accuracy for *Vibrio* spp. identification has not been reported. Fifty-five presumptive *Vibrio* spp. isolates were subjected to three diagnostic approaches: MALDI-TOF MS (Bruker Biosciences Corp.), conventional aerobic bacteriology with biochemical phenotypic testing, and DNA sequence analyses using three conserved genes, *rpoB*, *rpoD* and *toxR*. DNA sequencing was presumed to be the most accurate and speciation by this method was completed on all 55 isolates. There was moderate agreement between the sequencing data and MALDI-TOF MS; however, certain species such as *Vibrio ordalii*, were inaccurately identified by MALDI-TOF MS. Thirty-four isolates (61.8%) had MALDI-TOF MS scores >2.0, suggestive of accurate speciation and these included American Type Culture Collection (ATCC) strains of *Listonella (Vibrio) anguillarum*, *Vibrio alginolyticus*, *Vibrio vulnificus*, *Vibrio ordalii*, and *Photobacterium damselae* subsp. *damselae*. An additional five isolates (9.1%) were identified to the genus level with scores between 1.8 and 2.0. Two isolates (3.6%) produced scores <1.8 indicating inability to confidently assign genus or species. Fourteen isolates (25%) produced no score and thus no identification. It was also noted that the tube extraction method produced more consistent and higher quality scores compared to the direct transfer method. Conventional aerobic bacteriologic methods were applied to the same isolate

set, including consideration of the effects of added salt (with or without 2% NaCl incorporated in the media), temperature (15° vs 20°C), and incubation time (24 vs 48 hours). Speciation using conventional aerobic bacteriology was inaccurate and inconsistent, and dependent upon salt concentration, incubation temperature and time. However, ATCC strains were correctly identified provided the media included 2% NaCl, an incubation temperature of 15°C and 24 hours of growth. Only one clinical isolate, *P. damselae* subsp. *damselae*, was concordantly identified by both MALDI-TOF MS and conventional aerobic bacteriology. These findings indicate the MALDI-TOF MS database may benefit from data collected from additional *Vibrio* isolates in order to improve accuracy and permit confident use in *Vibrio* speciation of fish-origin.

CURRENT CATEGORY/DISCIPLINE: Bacteriology/Mycology

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