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**A multiplex PCR for simultaneous detection and differentiation of North American serotypes of bluetongue and epizootic hemorrhagic disease viruses**

**Imadeldin E. Aradaib, a, Wayne L. Smith, b, Bennie I. Osburn, b and James S. Cullor, b**

a-Department of Medicine, Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Khartoum, P.O. Box 32, Khartoum North, Sudan

b-Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA 95616, USA

In the present study, a multiplex RT-PCR-based assay for simultaneous detection and differentiation of North American serotypes of bluetongue (BT) virus (BTV) and epizootic hemorrhagic disease (EHD) virus (EHDV) in cell culture and clinical samples was developed. Two pairs of primers (B1 and B4) and (E1 and E4) were designed to hybridize to non-structural protein 1 (NS1) genomes of (BTV-11) and (EHDV-1), respectively. The multiplex PCR-based assay utilized a single tube-PCR amplification in which EHDV and BTV primers were used simultaneously in a multiplex format. The BTV primers generated a 790 base pair (bp) specific PCR product from RNA samples of North American BTV serotypes 2, 10, 11, 13 and 17; whereas EHDV serotypes 1 and 2 or total nucleic acid extract from non-infected baby hamster kidney (BHK) cells failed to demonstrate the 790 bp specific BTV PCR product. Likewise, the EHDV primers produced a 387 bp specific PCR product from RNA samples of EHDV serotypes 1 and 2, but not from BTV serotypes 2, 10, 11, 13, 17 or from total nucleic acid extract of BHK cell controls.

Two pairs of nested primers (B2 and B3) and (E2 and E3), internal to the annealing sites of primers (B1 and B4) and primers (E1 and E4), produced a 520 bp specific BTV and a 224 bp specific EHDV PCR product from BTV and EHDV first amplification products, respectively. These nested amplifications increased the sensitivity of the PCR assay and confirmed the specificity of the first amplified EHDV or BTV PCR products. The described multiplex RT-PCR-based assay could be used to facilitate rapid detection and differentiation of North American BTV and EHDV serotypes and to provide a valuable tool to study the epidemiology of these orbivirus infections in susceptible animal populations.

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**The Use of Pulsed Ultraviolet Laser (PUV) for the Cold Pasteurization of Bovine Milk**

**Wayne L. Smith, Manuel C. Lagunas-Solar, and James S. Cullor**

Dairy Food Safety Laboratory, School of Veterinary Medicine, Veterinary Medicine and Teaching Research Center, University of California, Davis, California 95616, USA

Crocker Nuclear Laboratory, University of California, Davis, California 95616, USA-<http://www.foodprotection.org/QuickLinks.htm>

Because of concerns that some potentially dangerous microorganisms may survive conventional heat pasteurization of milk and because the heat needed to sterilize milk affects marketability, the ability to efficiently cold pasteurize milk may become more desirable. In this pilot study, we investigated the use of pulsed ultraviolet (PUV) laser light to nonthermally (cold) pasteurize bovine milk.

Dairy bulk tank milk was treated with UV light (248 nm) emitted from a pulsed excimer laser. The samples were then analyzed for surviving bacteria by spiral plate counting and subculturing in Trypticase soy broth. Other bulk tank milk samples were inoculated with one of eight relevant milk bacterial species before being exposed to laser light. There was no growth observed for any of the plated or subcultured samples exposed to 25 J/cm<sup>2</sup>. One bacterial isolate was then used to inoculate milk to further investigate bactericidal laser light doses. Growth was observed for samples treated with an average of 0.3 to 6.6 J/cm<sup>2</sup> but not for those treated with 12.6 J/cm<sup>2</sup>. The results indicate that in principle, the bacterial content of milk can be adequately controlled by exposure to PUV laser light.

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### **Antibiotic susceptibility patterns for environmental streptococci isolated from bovine mastitis in central California dairies**

**P. V. Rossitto, L. Ruiz, Y. Kikuchi, K. Glenn, K. Luiz, J. L. Watts and J. S. Cullor**

Department of Population, Health, and Reproduction, Veterinary Medicine Teaching and Research Facility, School of Veterinary Medicine, University of California, Davis 93274, USA. [prositt@vmtrc.ucdavis.edu](mailto:prositt@vmtrc.ucdavis.edu)

Environmental streptococci are frequently isolated from bovine mastitis in dairy cows with only limited information available on the antimicrobial susceptibility of these organisms. A total of 362 environmental streptococci isolated from cases of bovine mastitis from the central San Joaquin Valley of California over a 3-yr period were used in the study. Overall, 39.9% of the strains tested were *Streptococcus uberis*, 42.2% were *Streptococcus dysgalactiae*, and 11.1% were *Enterococcus* spp. The antimicrobial susceptibility for these organisms was determined for the following antimicrobial agents: penicillin, ampicillin, cephalothin, ceftiofur, penicillin + novobiocin, erythromycin, pirlimycin, tetracycline, and sulfadimethoxine. Results demonstrate substantial differences in the susceptibility patterns for the various organisms collectively referred to as the environmental streptococci. The MIC<sub>90</sub> for penicillin was 0.06 microg/ml for

152 strains of *S. dysgalactiae* compared with 0.25 microg/ml for 133 strains of *S. uberis*. However, the *Enterococcus* spp. were the most resistant organisms tested. These data also indicate that the use of interpretive criteria based on human data may provide misleading results. In conclusion, these data confirm that the environmental streptococci are a diverse group of organisms comprised of several different genera and species and that identification of environmental streptococci to the species level is needed to appropriately modify control methods. Moreover, the use of the agar disk diffusion (Kirby-Bauer) susceptibility test for agents with human-based interpretive criteria is contraindicated, and these tests should only be performed with agents with mastitis specific interpretive criteria.

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### **Cytokines Gene Expression Patterns of Bovine Milk During Middle and Late Stages of Lactation**

**A.M. Alluwaimi ; J.S. Cullor**

The cytokine mRNA profiles of the bovine mammary gland were investigated using newly developed TaqMan® real-time polymerase chain reaction systems (Applied Biosystems, Foster City, CA, USA). Transcriptional activity of six cytokines, interleukin (IL)-2, IL-6, IL-12, interferon (IFN)-, tumour necrosis factor (TNF)- and granulocyte-macrophage colony stimulating factor (GM-CSF) was studied during the mid- and late-lactation stages. Transcripts for TNF-, GM-CSF, and IFN- were detected in all samples of both stages. However, IL-12 was only detected in 80 and 58 % of late- and mid-lactation samples, respectively. IL-12 expression was up-regulated in late lactation in comparison with the corresponding level in mid-lactation. The cytokines interaction in late lactation was more co-ordinated and their transcriptional levels were significantly correlated among each other, whereas, in mid-lactation significant correlation of the cytokines transcription was only seen with the TNF-, GM-CSF, and IFN-. Cytokine mRNA profiles between mid- and late lactation showed significant differences, which can be attributed to the dramatic changes that the mammary gland is subjected to during late lactation. The significant elevation of IL-12 transcriptional activity in late lactation and its relevance to the mammary gland immunity is discussed.

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### **Escherichia coli infections deriving from milk contamination.**

**Cullor, J.S., Smith, W.L., Selim, S.A., Rossitto, P.V.**

Proceedings of the Animal Health Conference, IDF World Dairy Summit 2001, Auckland, New Zealand.

Escherichia coli 0157:H7 infections resulting in human illnesses with serious consequences have established a strong association with the consumption of contaminated food of animal origin. The existence of this pathogen in the farm environment, apparently without any associated clinical symptoms in one of its natural reservoirs, cattle, causes significant on-farm food safety problems in controlling this foodborne pathogen. The presence of E. coli 0157:H7 in raw milk and dairy products is an indication of transitory animal infections and probable cross contamination in the processing plants or thereafter in the food chain.

Escherichia coli is a common bacterial inhabitant of the gut of wildlife, farm animals and humans. Hundreds of strains of E. coli are harmless to immunocompetent humans and animals; however, some may cause severe foodborne or waterborne disease. Enterohaemorrhagic Escherichia coli (EHEC) are a group of pathogens that cause haemorrhagic colitis in humans and animals, and may result in life-threatening complications in young children and the elderly. Escherichia coli 0157:H7 is counted among those EHEC considered dangerous when consumed by humans. Clinical signs of illness in humans caused by EHEC usually appear about three days after exposure but may occur within a range from 1 to 9 days. The clinical signs include abdominal cramps, watery diarrhea that can develop into bloody diarrhea (haemorrhagic colitis). Fever and vomiting may also occur and most patients recover within 10 days. However, in young children and the elderly, the infection can result in life-threatening complications, such as hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP). HUS is characterized by acute renal failure, hemolytic anemia and thrombocytopenia. It is estimated that up to 10% of patients with EHEC infection may develop HUS, with a case-fatality rate ranging from 3% to 5%. The toxins produced by EHEC are referred to as Verocytotoxins or Shiga-like toxins because of their similarity to the toxins produced by Shigella dysenteriae type 1. Strains of E. coli that can produce such toxins are also called STEC (Shiga toxin-producing E. coli) or TEC (Vero cytotoxin producing E. coli). There seems to be no international consensus regarding how this group of organisms is named at this time. Microbiological identification of the nearly 300 different serotypes of E. coli is accomplished by distinguishing between specific compounds that are found on the surface of the bacteria (somatic antigens or O-antigens) and on hair-like appendages (flagella or H-antigens) that extend from the bacterial cell surface. At this time, there have been 173 different O antigens and 56 different H antigens identified on various E. coli strains. It should be noted that although E. coli 0157:H7 infections are more frequently reported by diagnostic laboratories around the world, there are clear indications that other EHEC strains (called non-0157) are increasingly implicated in causing hemorrhagic colitis and HUS. The World Health Organization suggests that the 0157:H7 is the predominant serotype in the USA, Canada; United Kingdom and Japan. However, in mainland Europe, Australia and South America non-0157:H7 serotypes are more common in causing human illness. Human infections with EHEC occur via the fecal-oral route. This has been documented in person-person contact in day-care centers for children, homes for the elderly and some secondary transmission to family members. EHEC have been isolated from meat and meat products, fruits and vegetables, fruit juices, dairy products and water (drinking water and recreational waters).

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