

# 3<sup>rd</sup> Euro-Global Conference on Infectious Diseases

September 05-06, 2016 Frankfurt, Germany

## Detection of uncommon enteric bacterial pathogens from human diarrheal specimens by SYBR-Green real time PCR

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Acute diarrheal disease is still a major health problem and second most common cause of death worldwide in children under five years of age. Most of the morbidity occurs in low-income countries, where the etiologies and epidemiology of gastroenteritis remain incompletely understood. Diarrhea can be caused by a range of pathogens, including several bacteria. Conventional diagnostic methods, such as culture, biochemical tests and enzyme-linked immunosorbent assay (ELISA) are laborious and time consuming. We used SYBR-Green real time PCR assay targeting 10 uncommon diarrheagenic bacterial pathogens (*S. aureus*, Enterotoxigenic *B. cereus*, *C. perfringens*, *C. difficile*, *L. monocytogenes*, *P. shigelloides*, *Y. enterocolitica*, Enterotoxigenic *B. fragilis*, *A. hydrophila* and *P. alcalifaciens*) directly in fecal specimens from patients admitted infectious diseases hospital with acute diarrhea in Kolkata, India. The products formed were identified based on melting point temperature ( $T_m$ ) curve analysis. The assay was first validated with reference strains or isolates and exhibited a limit of detection of 103 to 105 CFU/gm of stool for each pathogen. A total of 1184 clinical fecal specimens from individual with diarrhea, previously cultured for enteric pathogens were evaluated. Enterotoxigenic *B. fragilis* was detected highest number about 80 (6.75%) followed by enterotoxigenic *B. cereus* 60 (5.06%), *C. perfringens* 46 (3.88%), *A. hydrophila* 45 (3.80%), *P. alcalifaciens* 44 (3.71%), *P. shigelloides* 39 (3.29%), *C. difficile* 39 (3.29%), *L. monocytogenes* 38 (3.20%), *S. aureus* 23 (1.94%) and *Y. enterocolitica* 14 (1.8%) respectively. We found SYBR-Green real time PCR assay for simultaneous detection of 10 target pathogens to be comprehensive, rapid, inexpensive and accurate, of high selectivity and is well suited for surveillance or clinical purpose.

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## Comparison of virological and serological findings on Moroccan bluetongue virus 1 and 4 infected sheep

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The bluetongue (BT) virus has been reported in Morocco in 2004. To investigate the involvement of BTV 1 and BTV4 infections, on immunity of sheep and to provide a basis for interpretation of serological and virological data, experimental infections were conducted with BTV-1 and BTV-4 strains. Antibody responses to BTV infections were evaluated using two enzyme linked immunosorbent assays and microtiter serum neutralization tests (mSNTs) in addition to virological monitoring based on RT-PCR. Large variation was observed between the three groups in clinical signs, showed variation in immune responses between animals. Viremia for BT virus was readily detected in sheep following BTV-1 infection, but was not detected following exposure to BTV-4 in group B and C. The high manifestation of clinical signs caused by BTV1 serotype compared to those caused by BTV4 could likely be due to BTV strains antigenicity and could probably be responsible in suppressing or manifesting BT symptoms and viremia for this serotype.

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