

RESEARCH NOTE

EVALUATION OF THE QUANTITATIVE BUFFY COAT ANALYSIS (QBC®) SYSTEM FOR THE DETECTION OF LEPTOSPIRA IN HUMAN BLOOD

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INTRODUCTION

Leptospirosis is an acute febrile illness transmitted to humans via exposure to urine of domestic and wild animals. In Hawaii leptospirosis has traditionally been an occupational disease affecting farmers, plantation workers, veterinarians, abattoir workers and military troops. Two regions of the State account for the majority of leptospirosis cases. The annual incidence rate in these areas is 11.9/100,000 population compared with 1.9/100,000 population for the State. Risk factors important in the transmission of leptospirosis in Hawaii are the use of water catchment systems in the home, the presence of skin abrasions during the exposure period, contact with cattle or cattle urine and the handling of animal tissue (Sasaki *et al*, 1993).

Diagnosis of leptospirosis is based on clinical findings and exposure history, positive blood/urine cultures and/or positive serology. Due to the protean nature of the disease, leptospirosis is commonly misdiagnosed as aseptic meningitis, fever of unknown origin or a viral illness (Martone and Kaufmann, 1979). Culturing of leptospirosis from body fluids can take as long as 6 weeks for detection while serology is largely limited to the confirmation of the infection during the convalescent stage of the disease. Due to the unique problem leptospirosis poses to Hawaii, we investigated the Quantitative Buffy Coat © Analysis System (Becton Dickinson, Franklin Lakes, New Jersey, USA) as an alternative means for the rapid

diagnosis of leptospirosis. The QBC © system, developed for the detection of blood parasites, was used without modification. Briefly, blood was added to capillary tubes precoated with acridine-orange, a cylindrical float was inserted into the tube and the unit was spun in a hematocrit centrifuge. The float compresses the blood cells against the wall of the tube. Erythrocytes, monocytes, lymphocytes, granulocytes and platelets separate into distinct bands clearly visible under a fluorescent microscope using a 50x oil objective. The QBC© system has been used to successfully detect malaria parasites, trypanosomes and microfilariae in infected blood (Spielman *et al*, 1988; Levine *et al*, 1989; Long *et al*, 1990). In our hands, the leptospirosis appear in the region of the platelet plasma interface. This region is quite bright and the contrast was improved with the addition of Trypan blue to the blood; final concentration 0.01%. The leptospirosis appear as thin white threads, many with hooked ends.

Whole blood, collected in EDTA, was spiked with *Icterohaemorrhagiae copenhageni* from a 4 day EMJH broth culture (Difco Laboratories, Detroit, Michigan, USA). Ten serial 2-fold dilutions were made starting at 5,120 organisms per milliliter. Two QBC © tubes were set up per dilution. In addition, two EMJH semi-solid culture tubes were inoculated with 75 µl of spiked blood from each dilution. This inoculum was approximately equal to the volume of blood in a QBC © tube. One tube was inoculated with 256 organisms directly from the broth culture as a control. The EMJH culture tubes were incubated at 28°C in the dark. The tubes were checked daily for the presence of Dinger's ring. Sixteen days after inoculation, the Dinger's ring was clearly visible in the control tube. Twenty-seven days after inoculation the

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EMJH culture tubes inoculated with 75 microliters from the 5,120, 2,560 and 1,280 organisms per ml dilutions were positive. Thirty-two days after inoculation, the tubes inoculated with 75 μ l from the 640, 320 and 160 organisms per ml dilutions were positive. The remaining EMJH culture tubes remained negative 60 days after inoculation.

The QBC[©] tubes were read blindly with approximately 5 minutes being spent on each tube. The leptospira could readily be detected at 5,120, 2,560 and 1,280 organisms per ml or 384, 192 and 96 organisms per QBC[©] tube. At 640 organisms per ml, only one of the duplicate tubes was positive. Organisms could not be found in the QBC[©] tubes at the remaining dilutions. Further experiments confirmed the sensitivity of the system to be approximately 1,000 organisms per ml of blood. In contrast, detection of leptospira directly in a drop of infected blood by dark-field microscopy has been estimated to require a concentration of greater than 20,000 organisms per ml (Diesch and Ellinghausen, 1975). The most effective strategy for the prevention of serious sequelae due to leptospirosis is early diagnosis and treatment. Doxycycline, when given early in the course of illness, can reduce the duration of the disease as well as alleviate the accompanying symptoms (McClain *et al*, 1984). Presently, however, there are no commercially available laboratory tests which can diagnose the disease in the first week of infection. The rapid diagnosis of leptospirosis using the QBC[©] system could therefore significantly improve patient care and treatment. This technique is currently being

evaluated in Brazil using clinically ill patients.

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