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Development and Evaluation of a Polymerase Chain Reaction (PCR)-Based Assay Using the 16S rRNA Gene for Detection of *Eperythrozoon suis* Infection

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Introduction

Eperythrozoon suis is a red blood cell parasite.¹ The disease, eperythrozoonosis, is characterized by four syndromes: 1) decreased reproductive efficiency of sows, 2) weakness and anemia in baby pigs with increased incidence of enteric and respiratory infections, 3) delayed production gains in feeder pigs, and 4) acute hemolytic anemia in feeder pigs.² Although the disease was first reported in the United States in 1932, our understanding of this organism and the disease it causes remains largely incomplete.³

Diagnosis of acute infection with *E. suis* is usually based on direct microscopic observation of organisms attached to red blood cells. However, this method has limitations because the presence of organisms in acutely infected pigs is transient and in chronically infected pigs very few organisms are found.¹ Serologic tests for the diagnosis of eperythrozoonosis have also been reported. These tests have diagnostic limitations due to a marked variability in antibody response, as well as a failure to identify acutely infected pigs.^{4,5} The lack of an efficient test for diagnosing acute and chronic *E. suis* infection has resulted in tremendous controversy concerning the true impact of this disease in pig populations.

The objective of our research studies was to develop a PCR-based test for diagnosing eperythrozoonosis. To this end we determined the specificity and minimal detectable number of *E. suis* organisms in the blood of an infected pig using PCR to amplify fragments of the 16S rRNA gene. Future studies will attempt to answer questions related to the prevalence of *E. suis* in pig populations and establish the usefulness of this assay for detection of *E. suis* in pig tissues to be used for human transplantation.

Experimental Design and Results

Our hypothesis is that both sick pigs and pigs with unapparent infections caused by *E. suis*

can be detected using PCR amplification of a gene specific for this organism in a blood sample. The object of the following studies were to develop a PCR assay based on the 16S rRNA gene that will allow us to test this hypothesis.

1) The presence of appropriate bacterial DNA for amplification was confirmed using universal primers. As shown in Fig. 1, we were able to amplify an approximately 1,500bp fragment of the 16S rRNA gene from a variety of genera belonging to the eubacterial group.

DNA extracted from blood of a pig experimentally infected with *E. suis* (Lane 1) during a parasitemic episode, as well as *Haemobartonella felis* (Lane 3), *Mycoplasma genitalium* (Lane 4) and *Bartonella bacilliformis* (Lane 5) gave bands of the expected size when they were amplified with this set of primers. When negative controls, DNA that had been extracted from a non-infected pig (Lane 2) or PCR reagents without template (Lane 6), were used as target substrates for PCR no amplification products were generated. M, 1kb-DNA size marker cut with EcoRI and HindIII.



Figure 1

2) Appropriate size fragments were purified using Wizard PCR Preps Purification System and cloned into pGEM-T™ Vector. Plasmids containing proper size inserts

were prepped and purified for sequencing using PERFECT prep Plasmid DNA Preparation Kit. Clones were sequenced by a dideoxy terminator method using Perkin-Elmer/Applied Biosystems automated sequencer at the University of Illinois' Biotechnologies Center, Genetic Engineering Facility. The 1394bp fragment of the 16S rRNA gene was sequenced in both the sense and antisense directions.

Submission of sequence data to Genbank

Locus AF0 29394 1374bp DNA 12 Nov 1997
Definition *Eperythrozoon suis* 16S rRNA gene, partial seq.
Taxonomy Eubacteria; Firmicutes; Low G + C gram-positive bacteria; Mycoplasmas and walled relatives; *Eperythrozoon suis*
Authors Messick, JM, Cooper, SK, and Huntley, MR
Comment Messick (1997) Recent sequence data (16S rRNA gene) from this laboratory suggest that *Eperythrozoon suis* is a novel mycoplasma which is distinctly positioned in the *Mycoplasma pneumoniae* pylogenetic group.

3) Based on the sequence data from the 16S rRNA gene, primer sets for species specific amplification were designed. The specificity of the these primers sets were examined. An example of the specificity of one of these primer sets is shown in Fig. 2.

An expected 1394 bp fragment of the 16S rRNA gene was amplified from DNA of an *E. suis* infected pig. No products were amplified when *H. felis* (Lane 3), *M. genitalium* (Lane 4) or *B. bacilliformis* (Lane 5) were used as target DNA. No product was amplified using DNA extracted from the blood of a non-infected pig (Lane 2) or using PCR reagents without target DNA (Lane 6).



Figure 2

was between 57 to 800. Therefore using PCR we were able to detect as few as 1 organism in 1,941 to 27,272 red blood cells.

Results of agarose gel electrophoresis of 1394 bp product amplified from 5-fold serial dilutions of PCR preparation. Lanes 1-7: PCR preparation was diluted 1:50, 1:250, 1:1,250, 1:6,250, 1:

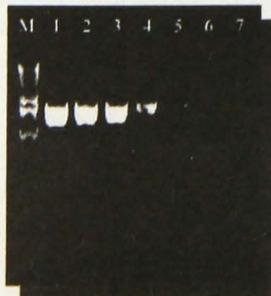


Figure 3

4) Using the above *E. suis* specific primer set, the expected 1394bp fragment was detected out to a dilution of 1:156,250 (Fig.5, Lane 6). Based on calculations, the minimum number of detectable organisms of the PCR assay

31,250, 1:156,250, and 1: 781,250. M, 1kb-DNA size marker cut with EcoRI and HindIII.

Application

This PCR-based assay is rapid, sensitive and specific for the detection of *E. suis* infection in pigs. In addition, the assay is easy to perform and lends itself to routine analysis. The diagnostic potential of this assay needs to be further evaluated in an experimental setting. Whereas, in the farm setting we will attempt to answer questions related to the prevalence of *E. suis* in pig populations, the risk factors associated with positive PCR assay results, and transmission and control of this disease. By testing seedstock, we can identify infected pigs before they are introduced into a clean herd. The PCR-based assay will serve not only as a tool to help us better understand the role *E. suis* plays in diseases of pigs, but will be of tremendous value in the testing pig tissues for the presence of this organism before they are used for human transplantation.

References

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