DIRECT DETECTION OF *STREPTOCOCCUS ZOOEPIDEMICUS* FROM ABORTED UTERUS OF MARES BY USING PCR TECHNIQUE

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ABSTRACT

Streptococcus zooepidemicus is one of the the main causitive opportunistic pathogen of the equine genital system and one of the secondary bacterial disease that causes mucosal bacterial infections . In the present study, PCR technique was used to detect *S. zooepidemicus* directly from uterine samples of infected mares based on specific amplification superoxide dismutase (sodA) gene primers that were designed by using the partial sequence of *S.equi subsp. zooepidemicus* strain 65843 superoxide dismutase (sodA) gene, (GenBank: GU436869.1) and primer3 plus for PCR primer design. The results showed high prevalence detection of *S. zooepidemicus* (76%) positive uterine swab samples (38/50). This study was concluded that *S. zooepidemicus* is the one of silant pathogen that cause metritis in mares. PCR is very fast and specific tool used to detect of *S. zooepidemicus*.

INTRODUCTION

Streptococcus equi subspecies *zooepidemicus* is Gram positive bacteria, round cocci which are set as pairs or chains, facultative, anaerobic, catalase and oxidase negative, nonmotile, hemolysis design depend on the species of streptococcus this species ordered as mesophilic bacterial growth (1,2). Lancefield group C from

this bacteria reflected commensal and opportunistic pathogen of respiratory disease in equine and also cause uterine infection(3). It can also cause several diseases in a wide range of animal , human hoset(4,5). This bacteria differ from *S.. equi* by some biochemical responses as lactose and sorbitol fermentation but not trehalose fermentation(6). About 98% DNA series character with *S. equi.* it shows as great antigens see L and see M have only been confirmed in particular strains of *S. zooepidemicus*(7). Newly certain strain of *S. zooepidemicus* exhibition recognized superantigens Szef,SzeN and Szep(8). Pathogenesis used very inconstant M-like cell wall anchard surface protein Szep which found in all strains of *S. zooepiedmicus* at least in horses where it fixes fibrinogen and exhibites antiphagocytic action that damages host protection device(9). It can spread from animal to human and cause diseases in human as periodic cases. The main outbreak described qualified food-born bases of *S. zooepidemicus* after feeding unpasteurized dairy yields and the symptoms described are meningitis, Septicaemia, purulent arthritis, nephritis and endocarditis(10,11). In addition Purpura hemorrhagica can be seen after infection with *S. zooepidemicus* in horses(12). This bacteria is not hostly controlled nor restricted to the respiratory system and wound or joint infections. The current study were aimed to detect *S. equi* subspecies *zooepidermicus* in the uterus of local breed pregnant mares by using PCR technique.

MATERIALS AND METHODES

Samples collection

Fifty uterine swab samples were collected from infected clinically horses with endometritis after abortion in different local field in Al-Diwanyia province. The samples were put in transport media and then sent to laboratory of Zoonotic Diseases Unite in veterinary college of AL_Qadissyia university for bacterial isolation.

DNA genomic extraction of Bacteria

DNA genomic of bacteria was extracted from 1ml of transport media swabs by using (Presto^{TM} Mini gDNA Bacteria Kit, Geneaid. USA). Following the manifactrer trainers. The removed gDNA was then tested by Nanodrop spectrophotometer and then stored at 20 ^{o}C untile use.

Polymerase Chain Reaction (PCR)

PCR technique was achieved to detect *S. zooepidemicus* by specific magnification of superoxide dismutase (sodA) gene. These primes were intended from NCBI-GenBank published sequence of *S. equi* subsp. *zooepidemicus* strain 65843 superoxide dismutase (sodA) gene, partial cds (Genbank: GU436869.1) and primer3 plus design online. The primers were used to amplify 172bp fragment of extremely conserved regions of sodA gene in *S. zooepidemicus*. sodA-Forward primer (GCAGCAGCTATTGATGACGC) and sodA-Reverse primer (GCTTGCCCTCTGAGATTGGT) were provided by (Bioneer company . Korea). PCR master mix was prepared by using (AccuPower® PCR PreMix kit. Bioneer. Korea). The PCR premix tube comprises freeze-dried pellet of (Taq DNA polymerase 1U, dNTPs 250µM, Tris-HCl (pH 9.0) 10mM, KCl 30mM, MgCl2 1.5mM, stabilizer, and tracking dye). The PCR master mix response was equipped conferring to kit instructions in 20µl total volume by adding 5µl of purified genomic DNA and 1µl of 10pmole of forward primer and 1µl of 10pmole of reverse primer. The PCR premix tube by deionizer PCR water into 20µl and briefly mixed by Exispin vortex centrifuge (Bioneer. Korea). The reply was done in a thermocycler (Mygene Bioneer. Korea) by set up the following thermocycler conditions; initial denaturation at of 95 °C for 5 min; monitored by 30 cycles at denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s and then final extension at 72 °C for 5 min. The PCR yields were noticed by electrophoresis in a 1.5% agarose gel, stained with ethidium bromide, and visualized under UV transilluminator.

RESULT AND DICUSSION

soda gene was succeflty amplified by using PCR. Results showed 38 positive samples out of 50 samples 78% of sodA gene . positive results showed clear and sharp bands on agrose gel electrophoresis(figure 1).

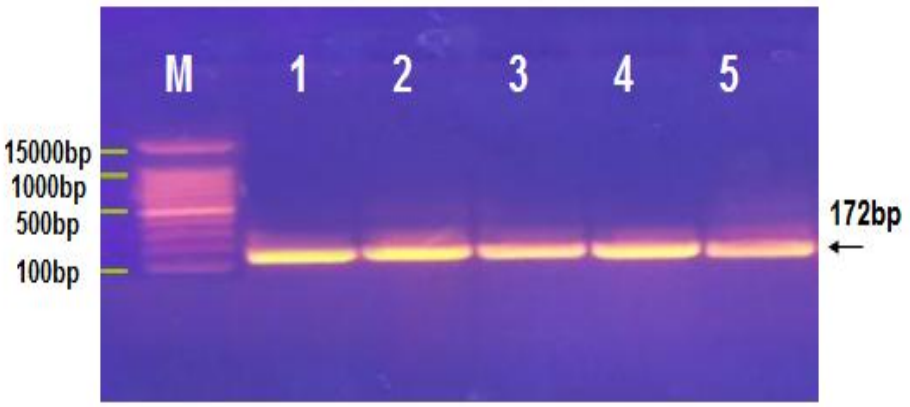

Figure (1): PCR products of DNA amplicon visualized by Ethidum bromide stained agrose gel electrophoresis are clearly detected Lane (M) represents DNA marker (100bp) , Lane (1) represents positive control DNA St. zooepidemicus isolate, and Lane (2-4) represents some positive samples at 172bp PCR product.

The PCR system has benefit to notice pathogen straight incompainion with bacterial culture method. Furthermore PCR is more subtle and can be used to notice together live and dead bacteria(13,14,15). In this study PCR was used for identification of *S. zooepidemicus* based on amplification of specific superoxide dismutase (sodA) gene, which is also used by Alber *et.al.* (16) who explained that extension of the sodA and seeI or seeH genes is recycled for detect and variation between *S. equi*, *S. equi zooepidemicus* and *S. pyogenes*. On other hand, Baverud and *et.al*(17) advanced the real time PCR technique as more profound and specific practice to magnify soda gene also in order to notice and distinguish of

Streptococcus spp. From the above , This study concluded that *S. zooepidemicus* is the one of saliant pathogen that responsible for metritis in mares and PCR technique is a very fast and specific technique to detect *S. zooepidemicus*.

التحري المباشر عن جرثومة العقدية السوافية Streptococcus zooepidemicus من رحم الافراس باستخدام تقنية تفاعل سلسلة البلمرة

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الخلاصة

تعد جرثومة العقدية السوافية واحدة من الممرضات الانتهازية الشائعة التي تصيب الجهاز التناسلي للفصيلة الخيلية وواحد من الامراض الجرثومية الثانوية التي تسبب التهابات الاغشية المخاطية (التهاب الانف والحنجرة) وربما تكون مسؤولة عن التسبب في تكوين الحالات المرضية الغازية الاكثر خطورة كالالتهاب الرئوي والالتهاب الرئوي الجنبي .في هذه الدراسة تم استخدام تقنية تفاعل سلسلة البلمرة لغرض الكشف والتحري عن جرثومة العقدية السوافية التي اخذت من عينات رحمية لافراس مصابة بالتهابات باستخدام التعاقب الذي صمم في هذه الدراسة sodAرحمية وتقوم هذه التقنية على التضخيم النوعي لجين) أظهرت نتائج GU436869.1 لعترة جرثومة العقدية السوافية 65843 (بنك الجينات sodaالجزئي لجين الدراسة الحالية ارتفاع نسبة الاصابة بالجرثومة قيد الدراسة (50/35) وبنسبة اصابة مقدارها 76% وخلصت نتائج الدراسة الى ان جرثومة العقدية السوافية هي واحدة من المسببات المرضية البارزة التي تسبب التهاب الرحم في الافراس وان تقنية تفاعل سلسلة البلمرة التي استخدمت في التحري عن الجرثومة تعد تقنية سريعة ونوعية في الكشف والتشخيص

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