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Research Article

Isolation of *Arcanobacterium (Actinomyces) pyogenes* from Abscessed Cattle Kidney and Identification by PCR

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Abstract: In this study, the presence of Arcanobacterium spp., which causes a variety of purulent infections involving the skin, joints and visceral organs, was investigated in abscessed kidney samples of cattle. A total of 500 cattle were examined at postmortem and 100 samples with abscess were collected. *Arcanobacterium pyogenes* was isolated in 40 (40%) of the samples examined.

DNA extracted from the isolates were amplified by Polymerase Chain Reaction (PCR) using specific primers derived from *plo* gene of *A. pyogenes* and all the isolates were determined to be positive by PCR. It was concluded that PCR employed in this study may be applied for identification of *A. pyogenes*.

Key Words: *Arcanobacterium pyogenes*, PCR, cattle, kidney

Apseli Sığır Böbreklerinden *Arcanobacterium (Actinomyces) pyogenes* İzolasyonu ve PCR ile İdentifikasyonu

Özet: Bu çalışmada, deri, eklem ve iç organlarda çeşitli prulent infeksiyonlara sebep olan Arcanobacterium spp.'nin apseli sığır böbreklerindeki varlığı araştırıldı. Toplam 500 sığır postmortem olarak muayene edildi ve apseli olan 100 örnek toplandı. *Arcanobacterium pyogenes* 40 (% 40) numuneden izole edildi.

İzolatlardan elde edilen DNA'lar *A. pyogenes*'in *plo* geninden türetilen spesifik primerler kullanılarak Polimeraz Zincir Reaksiyonunda (PZR) çoğaltıldılar ve tüm izolatların PZR'da pozitif olduğu saptandı. Bu sonuç çalışmada kullanılan PZR yönteminin *A. pyogenes*'in identifikasyonunda uygulanabilecegini göstermiştir.

Anahtar Sözcükler: *Arcanobacterium pyogenes*, PZR, sığır, böbrek

Introduction

Arcanobacterium (Actinomyces) pyogenes, recently reclassified from the genus Actinomyces on the basis of rRNA sequence, is considered to be a common inhabitant of the upper respiratory and genital tracts of domestic animals (1). Its isolation from the rumen of cattle (2,3) suggests that it may also be a common inhabitant of the gastrointestinal tract of these animals. *A. pyogenes* is also an important oppurtunistic pathogen, responsible for suppurative infections in a variety of domestic animals (4-6), avian species (7-9) and man (10,11). The involvement of *A. pyogenes* in economically significant diseases and syndromes such as liver abscess (5), mastitis (12), abortion and infertility (13) and postpartum uterine infections (14) ranks it as one of the most important bacterial pathogens of cattle. *A. pyogenes* also causes osteomyelitis in turkeys (8), and pneumonia in deer (15), and bighorn in sheep (16).

The organism enters the blood stream and causes septic arthritis, suppurative lesions and abscesses in various organs and tissues, mainly in the lungs (17). A pyogenes is often isolated from the abscesses in the lungs of ruminants, pigs and sometimes people (18,19).

The purpose of this study was to isolate *A. pyogenes* from abscessed cattle kidney and to identify the isolates by conventional methods and Polymerase Chain Reaction (PCR).

Materials and Methods

Material

A total of 500 cattle were examined for the presence of abscesses in kidneys at a local abattoir in Elazig province located in the East of Turkey, and 100 samples were collected. The samples were placed into separate sterile plastic bags. The kidney samples were immediately transported to the laboratories in a cool thermos and were processed for culture.

Bacterial Isolation

Abscessed cattle kidneys were inoculated onto blood agar (Merck, Darmstad, Germany) supplemented with 5% defibrinated sheep blood with a sterile loop, and were incubated for 48 hours at 37 ºC. Colonies that were small, circular, convex, opalescent with smooth glistening and surrounded with a narrow sharp hemolytic zone were picked up for further analysis. Pure cultures of isolates were prepared from the colonies that were beta-hemolytic, Gram positive and small curved rod-shaped in the microscopic examination. Routine biochemical tests, including catalase, oxidase, nitrate reduction, esculin, gelatin hydrolyzation, urease production, maltose, mannitol, sucrose or xylose fermentation, urease, Oxidation-Fermentation, were carried out to identify the isolates (20).

DNA Extraction

A few colonies from the suspicious cultures were picked up and suspended into an Eppendorf tube containing 300 µl distilled water. Bacterial suspension was incubated at 56 ºC for 30 min. Following this, the samples were treated with 300 µl of TNES buffer (20 mM Tris pH 8.0, 150 mM NaCl, 10 mM EDTA, 0.2% SDS, 200 µg/ml Proteinase K). Following 30 min of boiling step, the same amount of Phenol (saturated with Tris-HCl) was added to the suspension. The suspension was shaken vigorously by hand for 5 min and centrifuged at 11,600 g for 10 min. The upper phase was carefully transferred into another Eppendorf tube, and 0.1 volume 3 M Sodium acetate and 2.5 volume absolute ethanol were added to the suspension, and was left at -20 ºC overnight. After the precipitation stage, the suspension was centrifuged at 11,600 g for 10 min and upper phase discharged.

The pellet, obtained following the centrifugation at high speed for 10 min, was washed twice with 95% and 70% ethanol respectively, each step followed by 5 min centrifugation. Finally the pellet was dried and re-suspended in 50 µl of distilled water. A reference strain of *A. pyogenes* NCTC5224 (ATCC 19411) (Kindly gifted by S.J. Billington) was subjected to the extraction procedure as described above.

PCR

The PCR was performed in a Touchdown Thermocycler (Hybaid, Middlesex, England) in a total reaction volume of 50 µl containing 5 µl of 10x PCR buffer (10 mM Tris-HCl, pH 9.0, 50 mM KCl, 0.1% Triton®x-100), 5 µl 25 mM $MgCl_2$, 250 µM of each deoxynucleotide triphosphate, 2U of Taq DNA Polymerase (Fermentas, Lithuania) and 1 µM of primers (forward primer: 5'-GGC CCG AAT GTC ACC GC-3', reversed primer: 5'-AAC TCC GCC TCT AGC GC-3') derived from *pyolysin* gene *(plo)* of *A. pyogenes* and 5 µl of template sample DNA. PCR procedure was performed for 35 cycles consisting of 1 min denaturation at 94 °C, 1 min primer annealing at 55 °C and 1 min amplification at 72 °C, followed with a final extension step of 72 °C for 5 min (21).

The amplified PCR products were detected by ethidium bromide staining after electrophoresis in 1.5% agarose gels. For analysis, 10µl of the amplification products was loaded on 1.5% agarose gels with 5 µl 6x loading buffer (blue-orange dye, DW and Glycerol). Electrophorese power supply were run at 60 V for 1 h. The gel was stained with ethidium bromide solution (0.5 µg/ml) for 30 min at room temperature and was examined for the presence of 270 bp molecular size weight bands under ultraviolet transilluminator. The results were photographed on Polaroid Gel Cam (film type 667). For the estimating the band molecular weight of the positive PCR samples, 100 bp DNA ladder (Promega, Maddison, USA) electrophoresed with the samples on the same gel.

Results

Culture Results

Bacterial growth was observed in 51 of the 100 abscessed cattle kidneys after incubation at 37 °C for 48 hours. *A. pyogenes* suspicious isolates produced beta hemolysis zone around the colonies. Gram positive bacilli were observed at the Gram staining microscopy of the

isolates. Biochemical reactions of the isolates were typical of *A. pyogenes*. According to the results, *A. pyogenes* was identified from 40 (40%) of abscessed kidney samples. Remaining isolates were identified as *Staphylococcus aureus* (6%), *Escherichia coli* (5%), and Streptococcus sp. 2 (2%).

PCR Results

In the PCR examination, positive results with the molecular size of 270 bp were obtained from all *A. pyogenes* suspicious isolates. Expected amplification was obtained with the reference strains in each PCR trials (Figure)

Discussion

A. pyogenes is a common inhabitant of the mucous membranes of cattle, sheep, swine, and other economically important animals (4,5). *A. pyogenes* causes significant diseases and syndromes such as liver abscess (5). This agent is frequently isolated from a wide variety of pyogenic disease conditions in many species of domestic animals and in humans. Presumably, *A. pyogenes* occurs as a commensal pathogen on the mucous surfaces of warm-blooded animals (1).

In a study, *A. pyogenes* was cultivated from tail lymph nodes (61.1%), tail phlegmons (56.3%), abscesses (49.1%), epiphysiolyses (45.2%), liver abscesses (31.8%), panaritia at the beginning of fattening (20.5%) and aborted fetuses (14.9%). This organism was also isolated from nasal mucous membrane and retropharyngeal lymph nodes. The highest detection rate with 71.1% was obtained from the tonsils (22).

Bacterial contamination of 92 ewes with or without retention of fetal membranes was investigated by conventional bacteriological techniques. The principal bacteria isolated from the ewes with retention of fetal membranes were *A. pyogenes* (36% of the first sampling, 32% of the second) (23).

In this study, the presence of *A. pyogenes* in abscessed kidney samples were investigated by both conventional and molecular techniques. *A. pyogenes* was isolated from 40 (40%) of 100 abscessed cattle kidney samples. Other bacteria isolated were *S. aureus, E. coli,* Streptococcus sp. Our findings indicate that *A. pyogenes* was the predominant cause of kidney abscesses in cattle.

The isolation rate of *A. pyogenes* was obtained 3.6% from abscessed lymph nodes in a study performed in the same region (24). *A. pyogenes* isolation rate of this study was higher than that of the previous study. The studies were performed at different times and different materials were used in each study. It is also possible that the prevalence of *A. pyogenes* infections could be increased within this time. However, further studies are needed to reveal this possibility.

A. pyogenes was isolated from different organs and tissue except from kidney in many studies. So there is little information about the presence of this organism in

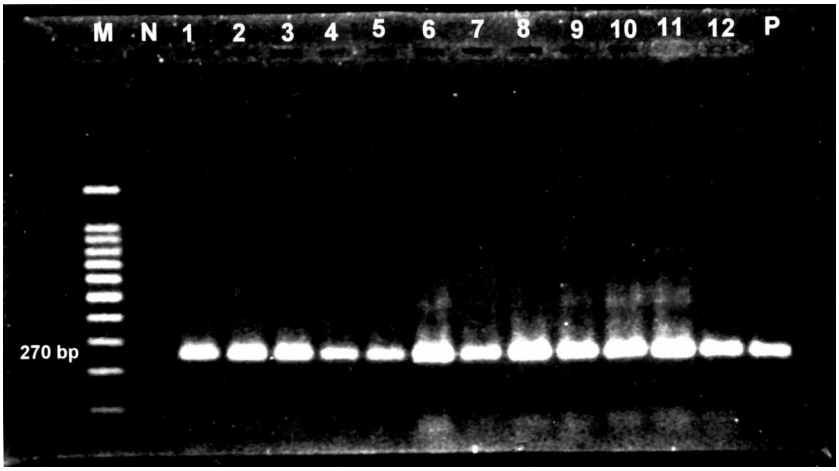

Figure. Agarose gel electrophoresis of PCR products obtained with purified DNA from *A. pyogenes* isolates (M: 100 bp marker, Promega, Maddison, USA, N: negative control, P: positive control, 1-12: *A. pyogenes* isolates).

kidney. This study indicated that *A. pyogenes* might cause kidney abscesses with high proportion (40%) in cattle.

With the development of molecular techniques used for the identification of veterinary pathogens, new procedures were being used for identification and confirmation of conventional identification results. Most used ones of these methods were PCR-based procedures. Current PCR research in the bacteriological laboratory focuses on the application of this technique to direct detection of pathogens in clinical samples. It is clear that this approach has several advantages over culture techniques for slowly growing or non-cultivable bacteria. Comparing of the PCR to the conventional identification procedures, PCR seems to be expensive and requires experienced research personnel. Biochemical tests used for the identification of many bacteria may give false results. Billington et al. (21) used a pair of oligonucleotid primers derived from *pyolysin* gene *(plo)* of *A. pyogenes* for identification of this bacteria by PCR. The same primer pairs were used for the amplification of *A. pyogenes* DNA in this study. All isolates identified by bacteriological methods as *A. pyogenes* yielded positive bands by PCR. Similarity of bacteriological and PCR techniques for identification was found to be 100%.

The isolation rate of *A. pyogenes* from abscessed kidney of cattle indicated that this organism may cause important economic losses in this region.

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