

## A Report on Sinuitis of Francolin

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**Abstract:** *Enterococcus faecalis* were isolated from Sinus of fossa orbitalis of diseased Francolin with sinuitis. The isolates were identified including morphological characteristics, culturing characteristics, physiological and biochemical characteristics. The results of the *16S rRNA* gene showed that the strains belonged to *Enterococcus faecalis*. Antimicrobial susceptibility tests showed that the strains were sensitive to Ciprofloxacin and Vancomycin.

**Key words:** Francolin, *Enterococcus faecalis*, isolation, susceptibility, vancomycin, China

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### INTRODUCTION

*Enterococcus faecalis* is widely distributed in nature. The organism has been isolated from domestic and wild animals and it is commonly found in water, soil and plant materials (Giard *et al.*, 2001). Conventional identification methods such as growth feature, biochemistry character and serological identification which are difficult. PCR has provided a means for the culture-independent detection of pathogen in a variety of clinical specimens and is capable of yielding results in just a few hours. PCR amplification of the *16S rRNA* gene was used to identify the isolated pathogen. Hence, a study was conducted to determine the isolates of pathogenic species in Sinus of fossa orbitalis of diseased Francolin with sinuitis and their confirmation by polymerase chain reaction amplification of the *16S rRNA* gene.

### MATERIALS AND METHODS

**Case history:** Disease outbreaks on one Francolin farm experiencing mortality were investigated. Francolin of age 50 days were affected. Mortality ranged between 0.05 and 0.2%. To begin, sick eyes of birds weeped and follow Sinus of fossa orbitalis bossed led to difficulty of food and drink. The course of disease was 20 days and pathogenetic condition was not control.

**Isolation of bacteria:** Samples of Sinus of fossa orbitalis of diseased Francolin were collected. Aerobic isolates were then selected by Luria-Bertani (LB) plates and blood agar after 24-48 h of incubation at 37°C. Colonies were picked at random and purified by restreaking on agar plates of the same media. Sugar fermentation tests

included dextrose, maltose, mannitol, inositol, melitose, xylose and sorbitol. Colony morphology, bacterial morphology and Gram stain reactions were recorded.

**Virus isolation:** Homogenized liver and spleen tissues were processed for virus isolation. Samples were inoculated on the chorioallantoic membrane of 9 day old embryonated duck eggs. Samples were considered negative if no lesions or death of embryos occurred after two blind passages.

**PCR amplification of the *16S rRNA* gene:** DNA was extracted and used as template for PCR. PCR amplifications of the *16S rRNA* gene were applied in this study by using the forward primer and reverse primer 1 with TaKaRa 16S rDNA Bacterial Identification PCR kit (TaKaRa Biotechnology (Dalian) Co., Ltd., China) according to the manufacturer's recommendations. The expected sizes of amplicons were approximately 500 bp.

**Sequencing of PCR products:** Aliquots were evaluated by agarose gel electrophoresis. The PCR products were purified by the use of TaKaRa Agarose Gel DNA purification kit Ver.2.0 (TaKaRa Biotechnology (Dalian) Co., Ltd., China) following the manufacturer's instructions and were cloned into pGEM T Easy Vectors (Promega Corporation, USA) for sub-sequent sequencing which was performed by Shanghai Sangon Biological Engineering Technology and Services Co., Ltd., of China. Sequence of 16S rDNA was submitted to BLAST alignment (<http://www.ncbi.nlm.nih.gov/BLAST>) against other 16S rRNA sequences available in Genbank.

**Identification of bacteria:** Tolerance tests included growth in 6.5% sodium chloride and at pH 9.6. Antimicrobial susceptibility tests were done on pure, 1 day old cultures of all the aerobic bacteria using the Kirby-Bauer disk diffusion test and Antimicrobial susceptibility slip (Shanghai Yihua Medicine Technology Limited Company, China) according to the manufacturer's recommendations. The following antimicrobials were tested: Vancomycin, ceftazidime, clindamycin, Tobramycin, ampicillin/sulbactam, imipenem and ciprofloxacin.

## RESULTS

**Isolation and identification of bacteria:** The cocci were isolated from Sinus of fossa orbitalis of diseased Francolin with ophthalmia. For species identification, the *16S rRNA* gene was amplified and sequenced. A 500 bp sequence was obtained and used for BLAST alignment. The isolates demonstrated 98% identity with *Enterococcus faecalis*.

The isolate produced acid from dextrose, maltose not fermented mannitol, inositol, melitose, xylose and sorbitol. The isolates produced  $\gamma$ hemolysis on blood agar medium. It grew in 6.5% sodium chloride and at pH 9.6. The isolate was resistant to ceftazidime, clindamycin, tobramycin, ampicillin/sulbactam, imipenem and sensitive to vancomycin and ciprofloxacin.

**Virus isolation:** No deaths or lesions were observed in chicken or duck embryos after two blind passages.

## DISCUSSION

The main habitat of enterococci is the intestines of warmblooded animals (Giard *et al.*, 2001) but because of their ability to survive heat treatment and adverse

environmental conditions they can be found in many foods of animal origin such as milk, cheese and fermented sausages (Franz *et al.*, 2003). *Enterococcus faecalis* have been recognized as pathogens of chickens and ducks (Peckham, 1966; Sandhu, 1988) but this was isolated from Sinus of fossa orbitalis of diseased Francolin that produced  $\gamma$ hemolysis. Francolin died due to an slowly sinusitis.

## CONCLUSION

Sinusitis of fossa orbitalis can be caused by *Enterococcus faecalis* and other bacteria such as *Escherichia coli* and infectious Coryza, isolation and identification of bacteria is essential for making a confirmatory diagnosis. In winter, the morbidity of sinusitis was very highly. The agent related to management and the result of medicate was not fine.

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