Molecular Detection of *Streptococcus Equi* (Strangles) From an Unorganized Stud Farm

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Abstract

Nasopharyngeal swabs collected from 12 horses (Eight with submandibular edema and 4 without any symptoms or lesion) brought to Madras veterinary college were screened for *Streptococcus equi* (*S.equi*) infection by culturing in Edwards medium base and PCR technique. Out of the 8 samples screened from the horses with submandibular lymphadenitis, 3 were found to be positive for *S.equi* and out of the 4 samples taken from apparently healthy horses, one was found to be positive for *S.equi* by culture and by PCR. The current study highlights the importance of initiating the regular screening process for strangles from both clinically affected as well as apparently healthy horses so as to prevent its spread in equine population and for initiating proper eradication programme.

Key words: Strangles, Horse, Polymerase Chain Reaction, Sub Mandibular Lymphadenitis

Introduction

Strangles in horses is caused by *Streptococcus equi*, which appears as gram positive cocci/coccobacilli in pairs or short chains, catalase negative and its inability to grow in 6.5% sodium chloride broth (Khoo et al., 2011). *S. equi* is a biovar or genovar of *S. zooepidemicus*, (Chanter, 1997). *S. equi*, which is highly adapted to Equidae family, demonstrates no serological variation, although genetic analysis demonstrates the existence of clones that vary geographically (AI-Ghamdi, 2000). There is variation in virulence that related to the amount of M protein and hyaluronic acid capsule produced (Timoney, 1993). It is a contagious disease of upper respiratory tract and associated lymph nodes. The disease is characterized by pyrexia, mucopurulent nasal discharge and abscessation of the lymph nodes of the head. It is one of the most frequently reported equine diseases worldwide and around 10% of cases may die from disseminated abscessation or purpura haemorrhagica (Chanter, 2000). The gold standard for diagnosis is bacterial culture of *streptococcus equi* on nasopharyngeal swabs, nasopharyngeal washings and guttural pouch lavage samples whereas PCR testing is more sensitive than bacterial culture (Sweeney et al., 2005). Moreover, rapid diagnosis is of great importance in case of strangles to prevent its quick spread to other...
horses, as disease has very high morbidity. Hence, PCR is a suitable technique for rapid screening of the suspected farms.

Key words: Strangles, horse, Polymerase chain reaction.

Materials and Methods

Nasopharyngeal swabs were collected from eight horses brought to Madras Veterinary College clinics from a nearby farm with a history of submandibular lymphadenitis. As the clinical signs were suggestive of Strangles, a contagious disease, farmers were advised to bring the rest of the horses for health checkup separately and swabs were collected from those four apparently health horses too. The swabs were subjected to culture in Edwards Medium Base, Modified (Hi-Media, Catalogue No M748) and allowed to grow for 24 hours. The organisms were picked and mounted in a slide and stained with gram’s stain as per the standard protocol (Sweeney et al. 2005). The swabs were later on used for DNA extraction using DNA extraction kit ((EZ-10 Spin Column DNA Gel Extraction Kit, Catalogue No, BS654, Biobasic Inc, Canada). Diagnostic PCR was carried out using published primers position in the genome of Streptococcus equi targeted by the primer sequence at n1-n1547 with the amplified product size of 1547bp as per the earlier description (Chanter et al., 2000). The cyclic conditions for PCR were initial denaturation at 95 °C for 2 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 54 °C for 2 min and extension 72 °C for 2 min, and then a period of 5 min of final extension at 72 °C using Taq PCR Master Mix (Red Dye) Catalog Number: BS9298, (Biobasic Inc, Canada). Amplified products were resolved in 1% agarose gel with 3kb DNA ladder Catalogue Number: GM 347 (Biobasic Inc, Canada).

Results and Discussion

Out of the 8 samples screened from the horses showing signs of submandibular lymphadenitis (Fig-1), 3 samples were found positive for Streptococcus equi and out of the 4 samples taken from apparently healthy horses one sample was found positive for Streptococcus equi by culture in Edwards Medium Base (Fig. 2a&b) (Sweeney et al. 2005) and also by PCR. The positive samples gave a product of 1547 bp on the analysis of PCR product (Fig-3). The detection of strangles from an apparently healthy horse indicates that the horse was carrier for the disease. Outwardly healthy horses have been recognized as a major factor in the transmission of the disease for decades. PCR is at least twice more sensitive than isolation on artificial medium for the detection of the organism (Newton et al, 2000). However, the diagnosis is presumptive not confirmative as PCR results only indicate presence of target nucleotide sequence however confirmation of disease can be achieved by demonstration of specific pathology of disease with isolation of causal organism and/or by proving Koch’s postulates. PCR can be used effectively to detect
asymptomatic carriers, to establish pre-transport or post transport status and to verify the success of treatment (Sweeney 2005). PCR is also used in the case of an outbreak to identify animals which may require further endoscopic investigation as suspect sub-clinical carriers (Newton et al. 1997).

Fig 1: Horse showing submandibular lymphadenitis

Fig 2a & b: Streptococcus spp. growth on Edwards medium and Gram’s staining

Fig 3: Gel picture revealing positive amplification of S.equi specific amplicon run against 3kb ladder

PCR is the most advanced molecular technique currently in use worldwide, having potential clinical applications, including specific or broad-spectrum pathogen detection, evaluation of emerging novel
infections, surveillance, early detection of bio threat agents, and antimicrobial resistance profiling. PCR-based methods may also be cost effective relative to traditional testing procedures which involves time consuming culture or serological procedures. The current study highlights that PCR as a highly sensitive time saving technique for screening of strangles. The study also emphasizes the importance of initiating the regular screening process for strangles from both clinically affected as well as apparently healthy horses by PCR, so as to prevent its spread in equine population for proper disease eradication programme.

References