

First Report of Isolation of *Fusarium solani* from Equine Keratitis in Gujarat, India

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Abstract

Mycotic keratitis, an infection of cornea due to fungi, is an important cause of ocular morbidity in humans and animals. In the present investigation, *Fusarium solani*, an opportunistic pathogen, is implicated as a primary etiologic agent of keratitis in a young male horse, which had a history of traumatic injury to left eye. The diagnosis was confirmed both by direct microscopy as well as cultural isolation. Cytological examination of smears prepared from corneal swabs demonstrated the presence of fungal hyphae by Giemsa technique. The pathogen was easily isolated in pure and luxuriant growth on Sabouraud agar and APRM (Anubha, Pratibha, Raj, Mahendra) medium. Detailed microscopic morphology of isolate in Narayan stain revealed hyphae, macroconidia, microconidia and chlamydospores characteristics of *Fusarium solani*. Retrospective epidemiological investigation indicated the prevalence of *F. solani* in the immediate environment of horse. It is emphasized that "APRM medium" and "Narayan stain", which are easy to prepare and less expensive than other stains and media, can be routinely used by researchers, particularly of poor resource nations, in diagnostic laboratory for the study of fungi including *Fusarium*. Early diagnosis and prompt therapy is highly imperative to prevent visual loss of the patient. As far as it could be ascertained, this seems to be the first report of involvement of *Fusarium solani* in equine keratitis from Gujarat, India.

Keywords: APRM Medium; Cytology; Equine; *Fusarium solani*; Keratitis; Narayan Stain

Introduction

Mycotic keratitis (fungal keratitis, keratomycosis) is caused by a wide variety of fungi such as *Aspergillus*, *Fusarium*, *Curvularia*, *Acremonium*, *Penicillium*, *Alternaria*, *Candida*, *Paecilomyces*, *Bipolaris*, *Exserohilum*, *Scopulariopsis*, *Cladosporium*, etc [1-4]. These fungi are widely prevalent in environment and are recovered from the soil, air, water, plant materials, and other natural substrates [5]. *Aspergillus* and *Fusarium* are the most important fungi responsible for one third of all traumatic mycotic keratitis [2]. There is over 60 species of *Fusarium* of which *F. solani* is incriminated in more than 50 % cases. Mycotic keratitis is frequently diagnosed in humans and rarely in animals, and is manifested by formation of a corneal ulcer, hypopyon, chemosis, oedema, and photophobia [2,3]. Fungi are unable to penetrate the intact cornea, and hence, trauma to cornea by plant material is attributed as the most important predisposing factor for the development of mycotic keratitis [2,6]. Mycological examination with direct microscopy and cultural isolation is imperative to confirm the diagnosis of mycotic keratitis [5]. It is pertinent to differentiate it from bacterial and viral keratitis. A plethora of topical antifungal drugs such as amphotericin B (0.15 - 0.3%), clotrimazole (1%), flucytosine (1%), fluconazole (1%), econazole (1%), itraconazole (1%), ketoconazole (1 - 2%), miconazole (1%), natamycin (5%), and voriconazole (1%) are used for the management of mycotic keratitis [3]. Therapy with topical corticosteroids should not be done as it may lead to poor prognosis. The management of keratitis due to *Fusarium solani* is challenging, as the pathogen shows resistance to several antifungal antibiotics [2]. Mycotic keratitis is a common condition in horses due to their large prominent eyes. The possible available literature did not reveal any information on mycotic keratitis due to *Fusarium solani* in

equines from India. Hence, the present communication elucidates the etiologic significance of *Fusarium solani* in keratitis of a young horse from Gujarat, Western India.

Materials and Methods

Two superficial corneal swabs collected on 10 December 2016 by the practicing veterinarian from the left eye of a 4-year-old male, non-descript horse, were submitted for the investigation. The animal belongs to a poor person of a nearby village of Bharauch, Gujarat, India. Cytological examination of smears prepared from clinical specimen was done by Giemsa technique to demonstrate of fungal agent. The isolation of the pathogen was attempted on Sabouraud dextrose agar with chloramphenicol and APRM medium (Anubha, Pratibha, Raj, Mahendra) medium. The later medium contained 2.0g of dried marigold flowers, 2.0 g agar, 50 mg chloramphenicol and 100 ml distilled water [7]. Suspected colonies were subcultured on APRM medium for further identification. Detailed microscopic morphology was done in Narayan stain, which contained 0.05 ml of 3% solution of methylene blue, 6.0 ml of dimethyl sulfoxide (DMSO) and 4.0 ml of glycerin [8]. The treatment regime included topical application of 1% itraconazole ointment two times daily on the affected eye of horse for about 8 - 10 weeks. The owner was advised to inform about the clinical efficacy of drug to the practicing veterinarian. In addition, three samples of soil were obtained from the animal shed and examined by dilution technique for the presence of *F. solani* [9].

Results

On clinical examination of horse, corneal ulcer of about 4 mm diameter was apparent in left eye, with lachrymation, chemosis, photophobia, and pain. The right eye appeared normal. The respiration, pulse rate and rectal temperature were in normal range. The animal owner narrated that horse received a minor trauma to the left eye with a soil contaminated object. Cytological examination of Giemsa stained smears revealed narrow, septate fungal hyphae. The culture on both mycological media yielded cotton like white growth at 30°C and later became bluish brown in color. No bacterial colony was observed on blood agar plate. The subculture of suspected colony on APRM medium, when examined under light microscope in Narayan stain showed thin slender hyaline hyphae, characteristic macroconidia, microconidia, and conidiophores, which confirmed the identity as *Fusarium solani* [10]. The animal owner did not report to the practicing veterinarian about the outcome of topical treatment with 1% itraconazole ointment. Therefore, the efficacy of this drug in fungal keratitis due to *F. solani* cannot be assessed in the present case. All the three soil samples collected from animal shed yielded several colonies of *F. solani* on APRM medium.

Discussion

The first case of mycotic keratitis in an agricultural farmer due to *Aspergillus glaucus* was recorded by Theodar Leber from Germany in 1879 [5]. Since then, cases of mycotic keratitis due to several saprobic fungi were reported in humans and animals from many countries of the world including India [3,4,6,11]. Among animals, maximum cases are encountered in horses [4,12-14]. In the present case, clinical observations, and laboratory findings conclusively established the causative role of *F. solani* in keratitis of a horse. The similar criteria of diagnosis was suggested by earlier investigators [1,4,6]. Ocular pain is the hallmark of in equine mycotic keratitis and the same was noticed in the present case. The role of trauma in mycotic keratitis has been described by several investigators [1,4,6] and the same was true in this horse, which received trauma with environmental object. This observation was further substantiated by recovering *F. solani* from the soil of animal shed. One can only speculate that if the animal would have acquired the infection from its environment following traumatic injury to eye.

The collection of corneal scrapings from affected eye requires technical expertise. It is very difficult to obtain corneal tissue for diagnosis in rural areas where veterinary hospitals are located. Hence, collection of superficial swab from corneal ulcer aseptically is advised to make the diagnosis of mycotic keratitis. The usefulness of corneal swab has been observed by Pal [15] who diagnosed a case of keratomycosis in a buffalo calf. However, the importance of corneal swab in diagnosing fungal keratitis should be further assessed by examining more number of cases.

It is advised that correct early diagnosis is highly imperative to start specific antifungal therapy of mycotic keratitis so that serious consequences can be prevented. It is deduced that cytology is a simple, reliable, inexpensive and rapid technique to diagnose fungal infection, and can be easily applied in field/remote areas where laboratory facilities for cultural isolation of fungi are lacking/non-existent.

Source of Support

Nil.

Conflict of Interest

None.

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