

---

## EQUINE HERPES VIRUS ASSOCIATED NUMMULAR KERATITIS IN A MULE - A CASE REPORT

RamaRaju Sagi S.B.<sup>1\*</sup>, Kirun Poodari<sup>2</sup>, Choubey S.K.<sup>3</sup> and Amit Kumar<sup>4</sup>

<sup>1</sup>Lab Officer, Central Military Veterinary Laboratory (CMVL), Meerut Cantt - 250001.

<sup>2</sup>Veterinary Officer, 6 Advance Field Veterinary Hospital (AFVH), Bareilly Cantt.

<sup>3</sup>Commandant, CMVL, Meerut Cantt., <sup>4</sup>CMVL, Meerut Cantt

\*Corresponding author: sbrsagi.831h@gov.in

---

### ABSTRACT

Equine Herpes Virus- 1 (EHV-1) is a widespread viral pathogen in equines causing significant mortality and affections like respiratory disease, abortions and devastating Equine Herpes Myeloencephalitis (EHM). The article presents PCR confirmation of EHV-1 from equine keratitis. The affected eye of the animal had nummular keratitis with coin shaped (Nummular) lesions on the corneal stroma. Nummular keratitis a feature of viral kerato-conjunctivitis in humans. More experimental studies are needed to understand the pathogenesis of EHV-1 induced equine ocular infections. To the authors' knowledge, this is the first ever clinical case report of EHV-1 from equine corneal disease from India.

**Keywords:** Equine Herpes Virus-1, Keratitis, Fluorescein test

### INTRODUCTION

Corneal affections are commonly observed in horses. They are sight-

threatening diseases requiring early clinical diagnosis, laboratory confirmation and appropriate therapy. Corneal affections of the equine eye may present initially as minor corneal epithelial ulcers or equine ulcerative keratitis with full-thickness corneal perforations. Complications like globe rupture, phthisis bulbi and blindness occur in untreated cases (Brooks, 2002). Several viral, bacterial and fungal pathogens have been incriminated in corneal disease in equines. Equine Herpes virus and Adeno viruses are considered to be viral causes of corneal diseases in equines. Few authors have reported Equine Herpes Virus (EHV) in equine ocular infections, especially in experimental models of EHV-1 infections (Hussey *et al.*, 2013 and HolzN *et al.*, 2019). EHV-1 associated chorioretinitis was first described in the late 1980's in llamas and alpacas and then in a mare and foal during a natural outbreak of paralytic EHV-1 infection (Whitwell *et al.*, 1992). The article presents detection of EHV-1 from an equine keratitis case.

**CASE HISTORY AND OBSERVATIONS**

A five year old mule with clinical lesions of keratitis was presented at 6 Advance Field Veterinary Hospital, Bareilly Cantonment, Uttar Pradesh. The animal exhibited slight pain with epiphora and photophobia in the left eye. The affected left eye had nummular keratitis with coin shaped (Nummular) lesions on the corneal stroma. The eye was subjected to fluorescein dye test. The animal showed multiple, round, sharply delineated, greyish white areas, throughout the cornea with presence of marked inflammation and vascularization in the eye with slight dye retention (Fig. 1). Corneal scrapings were collected from the case and were sent to Central Military Veterinary Laboratory (CMVL) for diagnosis.

Corneal scrapings were subjected to bacterial and fungal isolation. The samples did not yield any bacterial growth after 48 hrs of incubation or any fungal growth on enriched media after 5 days of incubation. The samples were further tested

for presence of EHV-1 to rule out Herpes virus related viral keratitis and the corneal scrapings were positive for EHV-1 on PCR (Fig. 2). The details of primers and PCR conditions (Gupta *et al.*, 1996) are given in Table 1.

**TREATMENT AND DISCUSSION**

As there is no approved treatment for EHV-1 in keratitis cases, the treatment may include anti-inflammatory drugs to alleviate pain and antibiotics to counter secondary bacterial infections. In the present case, supportive treatment with antibiotic (Neosporin ophthalmic solution) and NSAID (0.03 per cent Flurbiprofen eye drops) thrice daily was followed for two weeks and the animal showed improvement in clinical signs after three weeks of antibiotic treatment. Three weeks after the initiation of treatment, the corneal scraping of the animal was negative for EHV-1 on PCR. An anti-viral compound, Valacyclovir significantly decreased the viral replication and signs of disease in

**Table 1:** Details of PCR for EHV-1

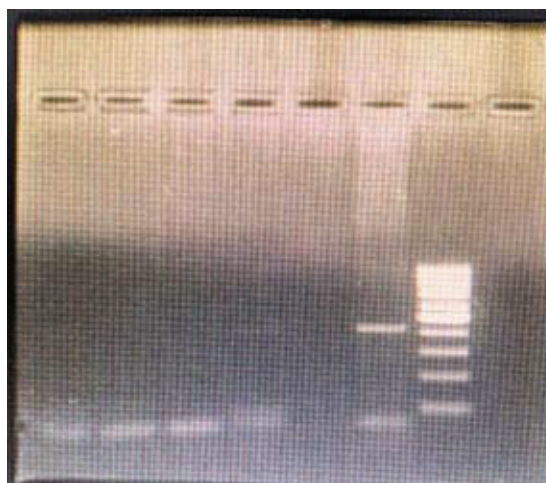
Sl. No:	Primer sequence (5' to 3')	PCR Amplification	PCR product
01	Forward primer: CTGTAGCATA GAATGGTACAGAGGA; Reverse primer: CCCC GCAAGTA ACGGCGATGATGC	30 Cycles and each cycle included DNA denaturation at 95° for 01 min, annealing at 60 °C for 01 min and extension at 72°C for 03 min	409 bp

EHV-1–infected horses in a study by Maxwell *et al.* (2017).

Pathogenic keratitis in horses may present with a mild, early clinical course, but require prompt diagnosis and therapy. Microbiological culture and sensitivity for bacteria and fungi are recommended for horses with rapidly progressing and deep corneal ulcers. In the present case report, bacterial, fungal and viral aetiologies were investigated. Viral keratitis is seen as a superficial punctate keratitis but is uncommon. Literature on ocular EHV incidence is limited especially in clinical cases (Hollingsworth *et al.*, 2015 and Holz *et al.*, 2019). Despite vaccination, EHV-1 is a widespread viral pathogen in the worldwide equine population causing significant mortality and affections like respiratory disease, abortions and devastating Equine Herpes Myeloencephalitis (EHM). It is postulated that EHM and ocular EHV-1 have similar pathogenesis. Frequency of ocular EHV-1 was determined to be 50-90 per cent following experimental infection by intranasal instillation (Hussey *et al.*, 2013). The term “nummular keratitis” was first used by von Stellwag in 1889 for the lesions which have a tendency to break down and ulcerate (Woods, 1946). Nummular keratitis, as observed in the present case, is a feature of viral keratoconjunctivitis in humans and represent an immune-mediated corneal stromal reaction



**Fig. 1.** Nummular keratitis (multiple, round, sharply delineated, grayish white areas) and vascularization of the affected eye. The eye lesion was positive for EHV-1 on PCR.



**Fig. 2.** EHV-1 PCR product of 409 bp

to viral antigens. EHV-1 is also known to cause chorioretinopathy presenting as permanent focal or multifocal “shotgun” lesions of the chorioretina in a substantial proportion of infected horses (Hussey *et al.*, 2013). More experimental studies are needed to understand the pathogenesis of

EHV-1 induced equine ocular infections. Though incidences of EHV in mules was reported, literature on EHV in ophthalmic cases is scarce (Mekonnen *et al.*, 2017 and Ataseven *et al.*, 2009).

Stringent quarantine and bio-security measures must be implemented immediately if EHV is detected in the farm, as there is risk of infection to other animals in the farm. For horses that develops fever with positive EHV-1 test, or having a high risk of exposure, use of anti-viral drugs may decrease the chances of EHM. Drugs like Acyclovir and ganciclovir were also found to be active against EHV-1, EHV-4 and EHV-3 *in vitro* (Vissani *et al.*, 2016). More research is required on the efficacy of these drugs, their cost effectiveness, and the optimal dosing regimen for EHV associated disorders.

## SUMMARY

The systematic approach to obtain resolution of the ocular infections requires appropriate clinical examination and laboratory diagnosis to initiate proper therapeutic regimen and prevent subsequent complications. This paper reports incidental detection of EHV-1 induced viral keratitis in a working mule. The occurrence of viral keratitis due to EHV-1 in working equines in India warrants more studies to be accurately commented upon with respect to its incidence, treatment and prognosis.

To the authors' knowledge, this is the first ever Indian case report of demonstration of EHV-1 induced viral keratitis in equines.

## ACKNOWLEDGEMENT

The authors are thankful to the Directorate General Remount Veterinary Services (DGRVS), Quartermaster General's (QMG) Branch, Integrated Headquarters of Ministry of Defence (Army), for providing the necessary facilities to carry out the work.

**Ethics statement:** This study does not involve animal experimentation and was conducted on cases reported in the hospitals, following standard operating protocols of animal handling and sample examination, upon informed consent of owners.

## REFERENCES

- Ataseven, V.S., Dagalp, S.B., Guzel, M., Bařaran, Z., Tan, M.T. and Geraghty, B. 2009. Prevalence of equine herpesvirus-1 and equine herpesvirus-4 infections in equidae species in Turkey as determined by ELISA and multiplex nested PCR. *Res. Vet. Sci.* **86**(2): 339-344.
- Brooks, D.E. 2002. Equine Ophthalmology. In: Proceedings of the Annual Convention of the AAEP. pp. 300-313

- Gupta, A.K., Singh, B.K., and Yadav, M.P. 1996. Application of polymerase chain reaction (PCR) for diagnosis of equine herpes virus-1 (EHV-1). *Indian J. Exp. Biol.* **34**(11): 1077-1080.
- Hollingsworth, S. R., Pusterla, N., Kass, P. H., Good, K. L., Brault, S. A., and Maggs, D.J. 2015. Detection of equine herpesvirus in horses with idiopathic keratoconjunctivitis and comparison of three sampling techniques. *Vet. Ophthalmol.* **18**(5): 416-421.
- Holz, C.L., Sledge, D.G., Kiupel, M., Nelli, R.K., Goehring, L.S. and Soboll Hussey, G. 2019. Histopathologic findings following experimental equine herpesvirus 1 infection of horses. *Front. Vet. Sci.* **6**: 59.
- Hussey, G.S., Goehring, L. S., Lunn, D.P., Hussey, S. B., Huang, T., Osterrieder, N., Powell, C., Hand, J., Holz, C. and Slater, J. 2013. Experimental infection with equine herpesvirus type 1 (EHV-1) induces chorioretinal lesions. *Vet. Res.* **44**(1): 118.
- Maxwell, L.K., Bentz, B.G., Gilliam, L.L., Ritchey, J.W., Pusterla, N., Eberle, R., Holbrook, T.C., McFarlane, D., Rezabek, G.B., Meinkoth, J. and Whitfield, C. 2017. Efficacy of the early administration of valacyclovir hydrochloride for the treatment of neuropathogenic equine herpesvirus type-1 infection in horses. *Am. J. Vet. Res.* **78** (10): 1126-1139.
- Mekonnen, A., Eshetu, A. and Gizaw, D. 2017. Equine herpesvirus 1 and/or 4 in working equids: seroprevalence and risk factors in North Shewa Zone, Ethiopia. *Ethiop. Vet. J.* **21**(2): 28-39.
- Vissani, M.A., Thiry, E., Dal Pozzo, F. and Barrandeguy, M. 2016. Antiviral agents against equid alphaherpes viruses: Current status and perspectives. *Vet. J.* **207**: 38-44.
- Whitwell, K.E. and Blunden, A.S. 1992: Pathological findings in horses dying during an outbreak of the paralytic form of Equid herpesvirus type 1 (EHV-1) infection. *Equine Vet. J.* **24**: 13-19.
- Woods, A.C. 1946. Nummular keratitis and ocular brucellosis. *Arch. Ophthalm.* **35**(5): 490-508.