**Bacterial and fungal co infection of ophthalmomyiasis due to Oestrus ovis near the coastal area of South India.**

**Abstract: Introduction:** Myiasis is the invasion or infestation of dipterian fly larvae in humans or animals. The involvement of any anatomical site of the eye is called as Ophthalmomyiasis. Since data on external ophthalmomyiasis is scarce in South India, the present study is carried out to explore the epidemiology, risk factors, clinical features and to determine the coexistence bacteria or fungi among the patients who were diagnosed as external ophthalmomyiasis.

**Materials and methods:** Patients with ophthalmomyiasis were prospectively identified over three years (January.2015 to December 2017). Slit-lamp microscopy was used to examine the conjunctiva. Larvae collected from conjunctiva were subjected to the lactophenol cotton blue staining technique. Two conjunctival swabs were collected from the patients who were diagnosed with ophthalmomyiasis. Standard microbiological methods were followed to identify the isolates and the antibiogram was determined by the disc diffusion method against bacterial isolates.

**Results:** A total of 19 patients were diagnosed as ophtholmomyiasis due to Oestrus ovis. Six out of 19 conjunctival swabs yielded microbial growth on culture media. Staphylococci were observed from three swabs. Growth of Pseudomonas aeruginosa and E.coli was also observed. Among 19 swabs inoculated on mycological media, two swabs yielded the growth and identified as Candida albicans and Aspergillus species.

**Conclusion:** Conjunctival swabs should be taken for the diagnosis of microbial co-infections infections and treatment modality changes according to microbiological diagnosis.

Keywords: Ophthalmomyiasis, Staphylococcus aureus, Plain forceps, Aspergillus

**Introduction:** Myiasis is the invasion or infestation of dipterian fly larvae in humans or animals.[1] Myiasis due to *Oestrus ovis (larva of bot fly)* is a well-known zoonotic disease that affects a variety of animals. Different parts of the human body may get effect due to myiasis infestation. Most commonly skin and body cavities (mouth, nostrils, ear and eye). The involvement of any anatomical site of the eye is called Ophthalmomyiasis.[2] Larvae, most commonly, attack the external surface of the eyes or ocular adnexia, e.g. the lids, conjunctiva or lacrimal ducts (external ophthalmomyiasis). In uncommon instances they may penetrate the eyeball itself (internal ophthalmomyiasis) or may involve the orbit (orbital myiasis).[3]

In external ophthalmomyiasis, the patient's typical history includes insect hit in the eye followed immediately by pain, burning sensation, lacrimation, foreign body movement in the eye, redness and the development of subsequent edema. Misdiagnosis is common, with an ascription of acute conjunctivitis to other causes. The clinical features may be mistaken for periorbital cellulitis or acute conjunctivitis.[4] *Oestrus ovis* myiasis is endemic in sheep and goats in tropical areas such as North Africa and South Asia. [5] Treatment is based on the removal of the larva followed by topical antibiotics, steroids and local analgesia. [6]

Since data on external ophthalmomyiasis is scarce in South India, the present study is carried out to explore the epidemiology, risk factors, clinical features and to determine the coexistence bacteria or fungi among the patients who were diagnosed as external ophthalmomyiasis. This analysis helps in guiding the appropriate selection of antimicrobial therapy and the prevention of superadded infections.

**Materials and methods:** This was a prospective observational study conducted at Vinayaka missions medical college and hospital, a tertiary health care center, over a period of four years (Jan 2015 to Dec 2018). Written informed consent was obtained from all patients. Institutional ethical committee clearance was obtained. All the patients who presented to ophthalmology department with the symptoms similar to conjunctivitis were evaluated for the presence of larvae. Relevant information about demographics, clinical features, treatment, and risk factors were recorded using standard proforma.

Slit-lamp microscopy was used to examine the conjunctiva. Two conjunctival swabs were collected from the patients diagnosed as ophthalmomyiasis. Standard microbiological methods were followed to identify the isolates [7] and to determine the antibiogram by disc diffusion method.[8]

**Results:** The epidemiological and clinical characteristics of infected patients were summarized (Table) A total of 19 patients were diagnosed as ophtholmomyiasis due to Oestrus ovis. Fifteen patients were males and the remaining four were females. Age was ranged between 21-56 years. All patients had insect hit while riding bikes or while walking along the sea coast which is close to the cattle grazing area. All patients had common symptoms such as redness, foreign body sensation, lacrimation and itching. Even pain was reported.

Ophthalmological findings: On slit-lamp examination, tiny and translucent worms, 1–2 mm in size, crawling over the bulbar conjunctiva were seen. The anterior chamber was found to be normal. Using 4% xylocaine drops as topical anesthesia, worms were removed manually with the help of sterile forceps. While removing worms mechanically with the help of forceps, they were tightly adhering to the bulbar conjunctiva with the aid of spines on their body. Plain forceps was used to remove the larvae from the conjunctiva. All the collected larvae from the conjunctiva were placed on a glass slide containing one drop of sterile saline and covered with a cover slip. Two conjunctival swabs were collected from the affected eye.

Microbiological findings: On microscopic examination, a spindle-shaped skeleton with multiple segments and intersegmental spine bands were seen. The larvae also showed a pair of sharp dark brown oral hooks and tufts of numerous brown hooks on the anterior margin of each body segment and they were identified as *Oestrus ovis.* [Figure.1] It was observed that the usage of toothed forceps might damage the morphology of larvae. [Figure.2]

Six out of 19 conjunctival swabs yielded microbial growth on culture media. Heavy growth of Staphylococcus aureus was observed from three swabs. Growth of Pseudomonas aeruginosa and E.coli was also observed. All bacterial isolates were found to be susceptible to commonly used ophthalmic antibiotics except P.aeruginosa which was sensitive to only amikacin and gentamicin. Among 19 swabs inoculated on mycological media, two swabs yielded the growth and identified as Candida albicans and Aspergillus species (Fig.3). (Table)

In all patients, treatment includes mechanical removal of larvae with the aid of plain forceps and application of topical antimicrobials with steroids. All patients responded to the treatment and no complications were noticed during follow up.

**Discussion:** In our study, over four years 19 patients were diagnosed with ophthalmomyiasis due to Oestrus ovis and 6 patients had superadded infection due to either bacteria or fungi. In our study insect hit was found to be the common risk factor especially while riding a motorcycle along the sea coast of the study region. All patients had similar symptoms, redness of eye, itching and foreign body sensation.

In 1840, Hope was the first person to describe myiasis. [9] Globally, human myiasis is distributed with various species and larger abundance in poor socioeconomic areas of tropical and semitropical countries. [3] Ocular involvement happens in less than 5 percent of all cases of human myiasis. [10] The most common cause of external ophthalmomyiasis is the larvae of sheep botfly, *Oestrus ovis*  Other agents involved in causing myiasis are *Rhinoestrus purpureus* ,  *Dermatobia. hominis* ,  *Chrysomya. bezziana* , *Lucilia* spp. , and *Cuterebra. [3]*

Morphologically adult bot fly resembles honeybee, which is yellow to gray brown, 10– 12 millimeters long. The gravid fly deposits larvae (Oestrus ovis) in or around the nostrils of cattle (host). These early stage larvae (first instar) deposited, adheres to the mucous membranes within the nasal cavities, then transform to second instars and crawl till the sinuses, where they develop further and mature into third instars, which are shed out for pupation under the soil. [11] The life cycle of this parasite is variable, from a couple of weeks to many months, depending on changes in the atmosphere. [12]

Man is an accidental host, with the eye being point of adherence for larvae.In man, the larvae cannot be alive beyond the early larval stage and are believed to die within ten days if not taken out. [12] Although the threat of orbital penetration and future serious consequences seems to below, it is prudent to get rid of the larvae from the mucosa of conjunctiva promptly.

External ophthalmomyiasis usually presents with ocular itching, foreign body sensation and a watery-mucopurulent discharge confined to the conjunctiva. [13] Draining of conjunctival sac with saline is ineffective in clearing out the larvae. Larvae hold the conjunctiva firmly with the help of tiny spines on its outer surface and anterior hooks. Possibilities of misdiagnosis as viral or allergic conjunctivitis is not uncommon. External ophthalmomyiasis typically happens in the summer and autumn which differs from another microbial conjunctivitis, which may occur throughout the year.

More scattered cases were reported since then from the Mediterranean area, like Italy, and also from Russia, Serbia (previous Yugoslavia), Africa, America, and Oman. [14.15]In a retrospective review of a 4-year period in Tunisia, O. ovis accounted for 2 of 65 identified parasitic infections of the eye. [16] There was also a case report involving an American soldier serving in northern Iraq. [17]

In this study, the cotton blue staining technique was used to view the larvae of O.*ovis*. Interestingly, an accidental finding was noted in the cotton blue mount technique, larvae of O.*ovis* which was simultaneously infected with Aspergillus *species.* Such finding of fungal infected O.*ovis* larvae from the human eye was not reported in the world literature to our knowledge.

The advantage of lactophenol cotton blue mount was quite obvious from the above findings. This helps to diagnose immediately and to institute the appropriate antimicrobial therapy for dual infection. The second advantage of the cotton blue technique, kills the parasite and able to view easily along with the fungus. By this method larvae could be preserved for more than two years without any disintegration.

**Conclusion:** A total of 19 patients were diagnosed as ophtholmomyiasis due to Oestrus ovis. Six out of 19 patients had co-infection due to bacteria and fungi. Our study highlights two things, the entire morphology and movements of O.ovis were visible when we used smooth forceps whereas morphology and movement of larvae got distorted when we used toothed forceps and conjunctival swabs should be taken for the diagnosis of superadded microbial infections and treatment modality changes according to microbiological diagnosis.

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**Table.**

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| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| S. no | Age/Sex | Season | No.of larvae | Co- infection | | Complaints | Treatment | Outcome |
| Bacteria | Fungi |
| 1 | 24/M | Summer | 20 | E.coli | - | FB, itching, lacrimation | Mechanical removal of larvae and broad spectrum antibiotic | Responded to treatment |
| 2 | 28/M | Early monsoon | 8 | - | - | FB,itching,  lacrimation | Mechanical removal of larvae and broad spectrum antibiotic | Responded to treatment |
| 3 | 33/M | Summer | 4 | - | - | FB,itching,  Lacrimation,Pain | Mechanical removal of larvae and broad spectrum antibiotic | Responded to treatment |
| 4 | 25/M | Summer | 6 | - | - | FB,itching,  Lacrimation | Mechanical removal of larvae and broad spectrum antibiotic | Responded to treatment |
| 5 | 35/M | Summer | 10 | - | - | FB,itching  Lacrimation | Mechanical removal of larvae and broad spectrum antibiotic | Responded to treatment |
| 6 | 21/M | Summer | 2 | S.aureus | - | FB,itching  Lacrimation,pain | Mechanical removal of larvae and broad spectrum antibiotic | Responded to treatment |
| 7 | 23/M | Summer | 5 | - | - | FB, itching,  Lacrimation | Mechanical removal of larvae and broad spectrum antibiotic | Responded to treatment |
| 8 | 31/M | Summer | 7 | - | - | FB,itching,Lacrimation | Mechanical removal of larvae and broad spectrum antibiotic | Responded to treatment |
| 9 | 42/M | Summer | 1 | - | - | FB,itching, Lacrimation | Mechanical removal of larvae and broad spectrum antibiotic | Responded to treatment |
| 10 | 31/M | Summer | 5 | S.aureus | - | FB, itching, Lacrimation | Mechanical removal of larvae and broad spectrum antibiotic | Responded to treatment |
| 11 | 53/F | Early monsoon | 2 | - | - | FB,itching | Mechanical removal of larvae and broad spectrum antibiotic | Responded to treatment |
| 12 | 21/M | Summer | 8 | - | - | FB, itching, lacrimation | Mechanical removal of larvae and broad spectrum antibiotic | Responded to treatment |
| 13 | 44/F | Early monsoon | 4 | - | Aspergillus | FB,itching | Mechanical removal of larvae , antibiotic and antifungal | Responded to treatment |

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| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 14 | 32/M | Early monsoon | 2 | - | - | FB,itching | Mechanical removal of larvae and broad spectrum antibiotic | Responded to treatment |
| 15 | 49/M | Summer | 5 | Pseudomonas species | - | FB, itching,Pain, | Mechanical removal of larvae and broad spectrum antibiotic | Responded to treatment |
| 16 | 33/F | summer | 6 | - | - | FB, itching,Pain,lacrimation | Mechanical removal of larvae and broad spectrum antibiotic | Responded to treatment |
| 17 | 23/M | Summer | >20 | - | - | FB, itching,lacrimation | Mechanical removal of larvae and broad spectrum antibiotic | Responded to treatment |
| 18 | 35/M | Summer | 2 | - | - | FB,itching,lacrimation | Mechanical removal of larvae and broad spectrum antibiotic | Responded to treatment |
| 19 | 56/F | Early monsoon | 8 | S.aureus | Candida albicans | FB,itching,lacrimation | Mechanical removal of larvae ,antibiotic and antifungal. | Responded to treatment |

FB: Foreign Body; S.aureus: Staphylococcus aureus

**Microbiological and clinical characteristics of 19 patients with ophtholmomyiasis due to Oestrus ovis**