Molecular diagnosis of *Mycoplasma conjunctivae* in an outbreak of infectious keratoconjunctivitis in sheep

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**Summary**

Infectious keratoconjunctivitis (IKC) is a painful, highly contagious ocular disease in sheep and goats. This study was carried out for identification and characterization of causative agent of ocular disease in a sheep flock consisting of 300 ewes in Mashhad, Iran. Several ocular swabs were taken from affected animals. The samples were pooled and processed in a laboratory for isolation of suspicious agent. Following inoculation of the pooled sample in PPLO broth and agar, turbidity and growth of colonies were observed in them, respectively. Sequencing of the 1013 bp PCR product of 16S rDNA gene revealed that the causative agent of the outbreak has 100% sequence identity to *Mycoplasma conjunctivae*. Based on our knowledge this is the first documented report of isolation and molecular characterization of *M. conjunctivae* in Iran.

**Key words:** Infectious keratoconjunctivitis, Sheep, PCR

**Introduction**

Infectious keratoconjunctivitis (IKC), also known as contagious ophthalma or pink eye, is an ocular disease in sheep and goats which is caused by *Mycoplasma conjunctivae* (Aitken Obe, 2007). *Mycoplasma conjunctivae* was first reported from an Australian sheep suffering from an ocular disease. This agent belongs to the *Mycoplasma neurolyticum* cluster of the *hominis* group, and is closely related to *Mycoplasma ovipneumoniae* (Giangaspero *et al*., 2010).

Purchasing and entering of animals such as a ram with mild or inapparent infections to a clean flock, is the main route of transmission of IKC between and within flocks. Wind in winter, and transportation of the animals are involved in the spread of disease. Greater intensity of sunlight and flies around the head and eyes can be effective in severity of the disease in summer (Akerstedt and Hofshagen, 2004; Aitken Obe, 2007; Scott, 2010).

Cost of care and treatment, pregnancy toxemia in heavily multigravid pregnant ewes secondary to inability of feeding due to blindness, are the major economic losses of the disease (Aitken, 2007; Scott, 2010).

Depending on the injury to the cornea and occurrence of unilateral or bilateral lesions, the blindness may be temporary/partial or persistent/absolute (Scott, 2010). When the cornea is involved, the disease may progress toward corneal ulceration. In sever cases, in addition to anterior uveitis, ulceration of cornea may progress to rupture of the anterior chamber, but the latter status is uncommon (Whitley and Albert, 1984; Scott, 2010). Two of the most important differential diagnoses include presence of foreign bodies within the conjunctival sac and entropions. Response to treatment is a good indicator for diagnosis of IKC (Scott, 2010).

**Materials and Methods**

In summer 2012, a sheep flock consisting of 300 ewes was visited in Mashhad, Iran. Ocular symptoms included eye inflammation, lacrimation, corneal opacity, and varying degree of blindness, mucoid to purulent ocular discharge, tear staining of the face, photophobia, blepharospasm either unilaterally or bilaterally in about 10 percent of the flock were observed in affected sheep. Mucopurulent nasal discharge was also seen in some involved animals. On close examination, conjunctivitis, with hyperaemic conjunctivae and injected sclera vessels were seen (Fig. 1a). Corneal opacities and keratitis were detected in a few head of the affected sheep, but there was no corneal ulcer in animals (Figs. 1b-d). On clinical examination, mild fever (40°C), decreasing ruminal movements and strength, and pulmonary harsh sounds were found. Blood samples were taken for complete blood count and determination of total protein and fibrinogen. Ocular swabs were collected from six severely affected animals and submitted to microbiology lab immediately. The samples were pooled and cultured for *Mycoplasma* spp. in PPLO broth and agar as described by Baas *et al*. (1977) and Bradbury (1998). DNA was extracted by using boiling method and the extracts were stored at -20°C till further use. Using genus specific primers, the polymerase chain reaction was performed; GPF; 5-GCT GGC TGT GTG CCT AAT ACA-3 and MGSO; 5-TGC ACC ATC TGT CAC TCT GTT AAC CTC-3 (Lierz *et al*., 2007; Ongor *et al*., 2011).