COMPARATIVE EVALUATION OF CONJUNCTIVAL AND AMNIOTIC MEMBRANE GRAFTS FOR THE MANAGEMEMT OF CORNEAL ULCERS IN DOGS

Thesis

Submitted to the Guru Angad Dev Veterinary and Animal Sciences University in partial fulfillment of the requirements for the degree of

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(Minor Subject: Veterinary Anatomy)

By

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CERTIFICATE – I

This is to certify that the thesis entitled, "**COMPARATIVE EVALUATION OF CONJUNCTIVAL AND AMNIOTIC MEMBRANE GRAFTS FOR THE MANAGEMEMT OF CORNEAL ULCERS IN DOGS**" submitted for the degree of **M.V.Sc.**, in the subject of **Veterinary Surgery and Radiology** (Minor Subject: **Veterinary Anatomy**) of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, is a bonafide research work carried out by **Gurpreet Singh (L-2015-V-77-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE – II

This is to certify that the thesis entitled, "**COMPARATIVE EVALUATION OF CONJUNCTIVAL AND AMNIOTIC MEMBRANE GRAFTS FOR THE MANAGEMEMT OF CORNEAL ULCERS IN DOGS**" submitted by **Gurpreet Singh (L-2015-V-77-M)**, to Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, in partial fulfillment of the requirements for the degree of **M.V.Sc.**, in the subject of **Veterinary Surgery and Radiology** (Minor Subject: **Veterinary Anatomy**) has been approved by the Student's Advisory Committee after an oral examination on the same, in collaboration with an external examiner.

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ABSTRACT

The present study was conducted on 28 eyes of 27 animals. After detailed signalment, neuro-ophthalmic, microbiological and cytological examination the animals were divided into three groups, based on the surgical technique adopted: **Group I (n=13)** comprised of bulbar conjunctival grafting, **Group II (n=4)** comprised of Amniotic membrane grafting and **Group III (n=11)** comprised of Surgical debridement. Group I was divided into two sub-groups i.e. Group I A (n=7) in which conjunctival pedicle grafting was performed and Group I B (n=6) in which conjunctival bridge grafting was performed. Group III (n=11) was sub divided into two groups i.e. Group III A (n=6) in which debridement with beaver blade was done and Group III B (n=5) in which debridement with sterile cotton swab was done. Brachycephalic breeds, primarily male pugs in the age group of 1-3 years were more prone to corneal ulceration. Menace reflex, pupillary light reflex, palpebral reflex and cotton ball test were the basic neuro-ophthalmic tests were used to confirm outcome of vision. Other specific diagnostic tests like Fluorescein dye test and Schirmer’s tear test were performed for confirmatory diagnosis of corneal ulceration and tear production respectively. Location based identification of corneal ulceration showed high percentage of centrally located corneal ulcers (60.61%). Culture sensitivity profiling revealed *Staphylococcus* spp as the most prevalent ocular microbe sensitive to tobramycin and neomycin antibiotics. Cytological findings were suggestive of inflammatory condition, with predominance of neutrophils. Topical tobramycin and carboxy methyle celluslose eye drops were used for post-operative management. In group I-A success rate of 85.71% was achieved with acceptable scar at ulcer bed. In group I-B four eyes (66.67%) regained vision. Graft dehiscence, corneal scarring and pigmentation were the common post-operative complications. In group II success rate of 100% was achieved with greater corneal transparency and minimum post-operative complications. In group III-A four eyes regained functional vision with overall success rate of 66.67%. Corneal scarring and pigmentation were the most significant post-operative complications. In Group III-B overall success rate of 80% was reported by this technique. Among conjunctival grafts, conjunctival pedicle grafting was better technique than conjunctival bridge grafting. Debridement with beaver blade offers corneal healing in short time period. It was concluded that human amniotic membrane grafting was better technique which offered rapid corneal healing with greater transparency.

Keywords: Corneal ulcer, conjunctival pedicle graft, Conjunctival bridge graft, Human amniotic membrane, Debridement with beaver blade, Debridement with cotton swab, Pug

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Signature of Major Advisor Signature of the Student

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LIST OF ABBREVIATIONS

%	:	Per cent
ºC	:	Degree Celsius
µ	:	Micro
µg	:	Microgroam
CBC	:	Complete blood count
dl	:	Decilitre
DLC	:	Differential leukocyte count
EDTA	:	Ethylene diamine tetra acetic acid
FGF	:	Fibroblast growth factor
Fig	:	Figure
AM	:	Amniotic Membrane
Hb	:	Hemoglobin
SIS	:	Small Intestine Sub Mucosa
TLC	:	Total leukocyte count

CHAPTER I

INTRODUCTION

Vision is a most valuable gift from the mother's nature to a living beings. In companion animals, it is of paramount importance for normal companionship behavior as well a normal well-being of the animals.

Outer layer of the eye ball is composed of white sclera and transparent cornea which play a vital role in vision. Cornea acts like a window for the entry of a light and image formation. Cornea is a structural and physiological boundary to the external environment that must fulfil unique requirements, including light refraction, transparency, and resistance against external hazards (Nautscher *et al* 2015). Anatomically canine cornea is composed of five different layers i.e. pre-corneal tear film, anterior epithelium, central stroma, Descemet's membrane and posterior endothelium. The stroma is composed of keratocytes, glycosaminoglycans and parallel bundles of collagen fibrils (Gelatt 1991).

Cornea is clear and transparent due to the non-keratinized nature of anterior epithelium, lamellar nature of the stromal collagen fibrils, lack of pigmentation and absence of blood vessels. Rich supply of sensory nerves to the cornea helps to preserve its transparency. Anything that interferes with normal stromal architecture, contributes to blood vessels migration from conjunctiva to cornea, increased corneal pigmentation or predispose to corneal edema which disrupts the cornea's transparency and results in corneal disease (Moore 2003). In corneal diseases, physio-anatomical parameters and functions get tampered resulting in loss of clarity of cornea and thereby loss of vision (Gelatt and Brooks 2011).

Good eye sight is an important part of well-being and a significant factor in retaining independence and quality of life in companion animals (Warren 2004). There are various eye affections which are responsible not only for decreased or complete loss of vision but can also lead to pain, discomfort and unpleasant appearance of the patient (Hartly *et al* 2006). Most common ophthalmic affections affecting vision are corneal ulcer, cataract, iris prolapse, proptosis, dermoid, glaucoma, cherry eye, keratoconjunctivitis sicca, lid lacerations, neoplasms of globe and adnexa.

Corneal inflammation or keratitis is one of the most common ocular diseases in both responsible for blindness and loss of the eye (Whitley and Gilger 1999; Ollivier 2003). Keratitis is classified into ulcerative and non-ulcerative (Whitley and Gilger 1999). Non-ulcerative keratitis in dogs is usually caused by mechanical irritation (pigmentary keratitis) or by immune-mediated process (pannus). Ulcerative keratitis or corneal ulcers can be of non-infectious (recurrent erosions, traumatically induced superficial ulceration) or infectious (bacterial, viral, mycotic) origin. Even in cases of originally non-infectious ulceration, after disruption of the epithelium secondary infection often occurs (Whitley 2000). Corneal ulcer is a break in continuity of corneal epithelium with or without loss of corneal stoma (Slatter 2001). In reference to the loss of corneal layers (Singh *et al* 2014), ulcers can be classified superficial, deep descemetocele and perforated corneal ulcer. Animals with corneal ulcers exhibit clinical signs of epiphora, pawing, blepharospasm, photophobia and corneal opacity (Jhanji *et al* 2011). Loss of vision may results from dense corneal opacification or corneal perforation secondary to deep ulceration. Variety of underlying causes includes trauma, chemical exposure, infection metabolic diseases or immune-mediated diseases (Hansen and Guandalini 1999). In addition, collagenases and other proteolytic enzymes cause progressive ulceration of corneal stroma with the risk of perforation. Continuous leakage of aqueous humor can result in swelling of the anterior chamber that leads to the anterior synechiae, glaucoma, cataracts, endophthalmitis and loss of vision. Therefore, prompt and appropriate treatment of corneal ulcers is extremely important to save vision. Higher incidence of corneal ulcers found in young to middle-aged dogs with age group of 1-3 years (Kim *et al* 2009; Ramani *et al* 2013).

Anatomical difference in the eye may predispose few dog breeds more prone to corneal ulceration (Moore 2003; Jose 2004; Ramani *et al* 2012). Higher incidence of corneal ulcers in brachiocephalic breeds e.g. pug could be due to protruded eye balls. Apart from this, hair projecting from the facial fold and relatively low corneal sensitivity could be the other attributing causes (Ramani *et al* 2012).

Diagnosis of ulcerative keratitis relies on clinical examination, cytology and microbiologic examination. Proper diagnosis is important for deciding line of treatment (Slatter 2001; Ollivier 2003). Schirmer’s tear test is the common diagnostic technique for identification of keratoconjunctvitis sicca and tear production. (Hartly *et al* 2006). Corneal ulceration and pathological changes to the epithelium surface can be tested using Fluorescein dye test and rose Bengal staining (Williams 2008).

Principles of corneal ulcer treatment include removal of the primary cause, reduction of inflammation, control of infection, enhancement of corneal healing, minimization of corneal scarring and replacement of lost corneal tissue (Gelatt 1991). While treating deep corneal ulcers, it is important to provide mechanical support to the weakened cornea with sound medical therapy (Boruchoff and Foulks 1990; Dawson and Sanchez 2015). Healing of the ulcer can be promoted by the use of tissue adhesives and a soft contact lens, suturing, conjunctival flaps and grafts (Severin 1995). Surgical therapy should be considered for deep corneal ulcers, recurrent corneal ulcers and stromal melting (Whitley 1991). In superficial corneal ulcers or epithelial erosions, keratotomy and epithelial debridement can be performed. Choice of surgical treatment varies according to size and depth of corneal defect (Wilkie and Whittaker 1997). Surgical techniques viz. conjunctival grafts (Boisjoly *et al* 1989, Dorbandt *et al* 2015), third eyelid flap (Helper 1981, Gelatt 1991), corneo-scleral grafting (Slatter 1990) and corneal transplantation (Severin 1995) have been advocated. Amniotic membrane provides rapid epithelialization of cornea without neovascularization and scarring (Vongsakul *et al* 2009). Multilayer cryopreserved human amniotic membrane (Kruse *et al* (1999); equine amniotic membrane (Lassaline *et al* 2005), canine amniotic membrane (Kalpravidh *et al* 2009), frozen fresh amniotic membrane (Agraval *et al* 2017) techniques have been suggested.

Keeping in view the high incidence and clinical importance of corneal ulcer, there is a need for further studies for their successful management. Therefore, the present study was planned with following objectives:

1. To evaluate the various types of conjunctival and amniotic membrane grafts for the management of corneal ulcers in dogs.
2. To evaluate the efficacy of surgical debridement in the healing of corneal ulcers in dogs.

CHAPTER II

REVIEW OF LITERATURE

A comprehensive review pertinent to the surgical management of corneal ulcer is presented under the following headings and sub headings.

Corneal ulcer

2.1 Corneal anatomy and Physiology

2.2 Corneal healing

2.3 Corneal defense mechanism

2.4 Corneal ulcer grading and classification

2.5 Incidence of corneal ulcer:-

- Age wise distribution
- Sex wise distribution
- Breed wise distribution

2.6 Causative factors and aetiology of corneal ulcers

2.7 Clinical signs associated with corneal ulcers

2.8 Diagnostic techniques for corneal ulceration

- Ophthalmic reflexes
- Schirmer's Tear Test
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- Diagnostic corneal cytology

2.9 Surgical management of corneal ulceration:-

- Conjunctival grafting
- Amniotic membrane grafting
- Surgical debridement

2.10 Complications associated with corneal ulcers

2.1 Corneal anatomy and Physiology

Startup (1984) studied avascular nature of cornea and found that avascular corneal was responsible for the decrease in corneal surface temperature by 0.5 to 10^{0} degree making it more susceptible to infections and ulceration.

Renwick (1996) reported that cornea comprised the one-fifth of fibrous tunics of the eye and its major characteristic includes avascularity, non-myelinated nerves, non-keratinized epithelium, a regular organization of collagen lamellae in corneal stroma and a relatively dehydrated state.

Robert *et al* (2001) noticed that cornea was highly differentiated tissue rich in extra cellular collagen matrix (ECM) that insures its dual functions i.e. transparency and protection of inner eye tissues. The precise regulation of the diameter and orientation of collagen fibers and inter fibrillar spaces showed interactions between glycosaminoglycan's and collagens synthesis. These interactions changes with age (Maillard reaction) and in several pathological conditions as corneal dystrophies and wound healing.

Abrams *et al* (2002) studied the epithelial basement membranes and endothelial (Descemet's) basement membranes of the canine cornea and concluded that epithelial and endothelial basement membrane surface was made up of an intricate meshwork of pores and fibers. Endothelial basement membrane was smaller in size than the epithelial basement membrane.

Maggs (2008) stated that there was no blood vessel in the normal cornea. However vascularization of the cornea was may be induced by different stimuli including stimulated lymphocytes or their elaborated lymphokine.

Alario and Pirie (2014) estimated the mean central corneal thickness (CCT) for normal canine eyes examined via spectral-domain optical coherence tomography (SD-OCT) and ultrasonic pachymetry (velocity set at 1636 m/s) was 587.72± 32.44 lm and 598.54± 32.28 lm, respectively.

Nautscher *et al* (2015) reported that cornea was composed of four layers: corneal epithelium, stroma, Descemet's membrane (DM) and corneal endothelium. The anterior corneal epithelium has three layer structure consisted of superficial non keratinizing stratified squamous cells (stratum superficiale), intermediate wing cells (stratum intermedium), and basal cells (stratum basale). Canine corneal epithelium consisted of three to nine cells rows with central, peripheral and limbal thickness 138.80 ±19.60, 121.50±16.30, 101.80±30 respectively.

2.2 Corneal healing:-

Fujikawa *et al* (1984) opined that the basement membrane components such as laminin, type IV collagen and bullous pemphigoid antigen (BPA) did not play a crucial role in corneal wound healing, whereas fibrin or fibronectin was indispensable for it.

Jester *et al* (1995) studied corneal wound healing in the rabbit eye and confirmed that corneal fibroblasts moved into the wound by as early as day three and replaced the fibrin mesh adjacent to the epithelium by day seven. There was a sequential positional change which oriented the cells and their interconnected processes, parallel to the wound margin.

Willeford *et al* (1998) reported that matrix protein like fibrin and fibronectin caused the epithelial cell to release the plasminogen activator. There was detachment of the newly formed scaffold when plasminogen got converted to plasmin and enabled the epithelial cells to adhere to the underlying basement membrane.

Whitley (2000) noted that uncomplicated superficial ulcers resolved in a few days, whereas refractory corneal ulcers required weeks or months to heal.

Miller (2001) found that corneal healing was completed when corneal epithelium adhered to the stroma and this took about one week if the basement membrane was not disturbed during the injury.

Bentley and Murphy (2004) categorized corneal wound healing into epithelial wound healing, stromal wound healing and endothelial wound healing and reported that after the initial lag phase of epithelial wound healing, the epithelial cells start migrating radially at a constant rate of approximately 20-50 um/h. Corneal wound healing was slowed down in case of a breach in the underlying basement membrane, which could take up to one year to heal if violated.

Netto *et al* (2005) opined that corneal epithelium, stroma, nerves, inflammatory cells, and lacrimal glands were the main tissues and organs which played a major role in wound healing. Keratocyte apoptosis and necrosis, keratocyte proliferation, inflammatory cell migration and myofibroblast regeneration were few of the processes which were studied.

Carter (2009) reported that fibrin, fibronectin and plasmin were important mediators in corneal restoration. Fibrin and fibronectin produced by basal epithelial cells and stromal keratocytes, acted as a temporary scaffold for new migrating epithelial cells and plasmin was responsible for cleavage of old epithelial cells attachment

2.3 Corneal defence mechanism

Gerding and Kakoma (1990) concluded that resident (commensal) non-invasive flora of the canine conjunctival sac and cornea inhibits the growth of pathogens by nutritional competition, occupying space and also by secretion of active substances that inhibit transient flora. Overall these transient floras were considered as important part of eye protection against infection.

Rieck *et al* (1992) reported that basic fibroblastic growth factors (bFGF) promotes proliferation of corneal epithelial cells and improves the quality of corneal wound healing. Basic fibroblastic growth factors (bFGF) facilitated recovery of corneal epithelial cells, shorten the closure time for corneal epithelial wounds, increase the density of corneal epithelial cells and accelerate the restoration of corneal epithelial cells.

Ohtani *et al* (1993) concluded that basic fibroblastic growth factors (bFGF) has potent anti-inflammatory activity by inhibiting the proliferation of lymphocytes in response to interleukin 1 (IL-1) and interleukin 2 (IL-2) and the production of cytokines and reduces antigen II expression.

Ollivier (2003) reported that regular sloughing of epithelial cells (turnover 5–7 days) precludes adherence of bacteria. Tight junctions of cells and basement membrane prevent further deep infiltration of bacteria. Tear film secretions contains several immune-active substances (lysozyme, β-lysin, lactoferrin, IgA) which provide protection to the eye.

2.4 Corneal ulcer grading and classification

Corneal ulcer classification

Mandell (2000) categorized corneal defects on the basis of their depth and opined that the treatment modality to be adopted depended on the depth of the ulcer.

Moore (2003) categorized corneal ulcers as superficial, deep and descemetocele according to the depth and complicated, uncomplicated, refractory and progressive based on the ease of healing.

Kecova *et al* (2004) stated that infectious and non- infectious were two categories of keratitis in humans, whereas in veterinary medicine, it was traditionally classified as ulcerative and non-ulcerative.

Superficial corneal ulcer

Mandell (2000) stated that the superficial ulcers were usually more painful since they involved a more innervated part of the epithelium.

Moore (2003) concluded that superficial corneal ulcers comprised of corneal epithelium and basement membrane wherein stroma may or may not be involved and was considered refractory if it did not heal within 5-7 days and complicated if it increased in size or depth.

Ollivier (2003) found that superficial ulcers which healed in a few days were usually not infected, however, antibacterial therapy was recommended to prevent growth of secondary, opportunistic bacteria since epithelial integrity was compromised.

Kim *et al* (2009) in a retrospective study of ulcerative keratitis in dogs noted that superficial ulcers healed without complication within 5-13 days when administered medical therapy.

Deep stromal ulcer

Mandell and Holt (2005) opined that deep stromal ulcers required vascularization for healing and took three weeks to heal.

Melting ulcer

Whitley (2000) successful managed the melting ulcers included controlling infection and reducing the impact of collagenase and other proteases on the cornea.

Ollivier (2003) stated that complicated corneal ulcers may be described as melting ulcers. When proteases, produced by microorganisms, inflammatory cells, corneal epithelial cells and fibroblasts, took an upper hand over its inhibitors, corneal destruction ensued.

Goulle (2012) described melting ulcers as gelatinised and liquefied corneal stroma where extensive debridement of necrotic and collagenolytic corneal tissue was done by keratectomy.

Indolent ulcers

Gelatt and Samuelson (1982) studied histology of epithelium of indolent ulcers, observed degeneration of basal epithelial cells, thick irregular basement membrane, and decreased number of hemidesmosomes which preventing adherence of epithelial cells to the stroma and resurfacing of corneal epithelium.

Morgan *et al* (1994) stated that non-healing (indolent) corneal ulcer was superficial epithelial defect of the cornea, without the involvement of stroma, bordered by non-adherent epithelium.

Willeford *et al* (1998) assessed proteolysis level in lacrimal fluid of dogs with normal cornea and with persistent corneal ulcerations and concluded that the elevated levels of proteolytic activity was the possible reason for the condition.

Whitley and Gigler (1999) studied that indolent ulcers, refractory epithelial erosions, recurrent corneal erosions syndrome, rodent ulcers was superficial corneal ulcers that heal slowly characterised by overlapping lip of non-adherent epithelium around the ulcers edge that stain positive to fluorescein stain and usually occurs bilaterally.

Moore (2003) reported that refractory ulcers were generally non- progressive resulting from failure of anterior epithelium to adhere to stroma, which was evident as an ‘epithelial lip’ at the edges, outlined by fluorescein stain. Proteinases like plasmin disrupted the fibrin or fibronectin matrix and metalloproteinase delayed basement-membrane replacement and adhesion which resulted in delayed healing and subsequently CCED.

Brunott *et al* (2007) stated that corneal debridement, grid or punctate keratotomy, thermal cautery of the cornea; superficial keratectomy, temporary tarsorrhaphy, conjunctival grafts or flaps were the treatment modality options for chronic epithelial defects in dogs.

Janssens (2007) concluded that fluorescein stain aids in the diagnosis of indolent ulcers by migrating under the loose flaps of the epithelium and stains the surrounding anterior stroma, and thus ulcer appear larger than the actual size.

Samuelson and Brooks (2011) stated that indolent corneal ulcers in brachycephalic breeds could be either primary or secondary to eyelash or eyelid abnormalities, infection, tear film abnormalities or corneal oedema.

Descemetocele

Wilkie and Whittaker (1997) stated that a deep corneal ulcer which did not retain fluorescein stain in the central portion was a descemetocele.

Hamilton (1999) described descemetocele as a protrusion of Descemet's membrane through the floor of an ulcer due to the pressure of aqueous humour behind it.

Featherstone and Stanley (2002) observed that descemetocele was deep corneal lesion characterised by complete destruction of corneal epithelium and stroma, leaving a lesion lined only by Descemet's membrane and corneal endothelium.

Mitchell (2011) observed that a clear area at the base of a deep defect in cornea after instillation of fluorescein dye indicated a descemetocele, since thedescemet's membrane did not take up the lipophobic stain.

Ledbetter and Gilger (2015) stated that descemetocele's were a surgical emergency owing to the potential for infection and intraocular inflammatory damage.

2.5 Incidence of corneal ulcer with respect to age, sex and breed

Age wise distribution of animals affected withcorneal ulcers.

Turner and Blogg (1997) reported different treatment techniques for the management of corneal erosions in dogs aged 11 months to 12 years with mean age of (6.5 years). Higher incidence of corneal ulceration was found in pure breed Boxers (60%) followed by Corgi cross (30%) and rest were mixed pure breed dogs. The incidence was found more in males (80%) than in females (20%).

Wilkie and Whittaker (1997) observed that older dogs and certain dogs breed such as Boxer, Golden retriever, Corgi, Miniature poodle appeared to be at high risk for development of corneal ulceration.

Murphy *et al* (2001) concluded that higher incidence of corneal ulceration in age group between 1-3 years was (50%) followed by 4-7years (29%) and above 8 years (21%).

Moore (2003) observed that indolent corneal ulceration was more commonly occurred in middle to older age dogs, with a mean age of 8.2 years.

Kim *et al* (2009) studied the incidence of ulcerative keratitis in dogs of age groups of 3 years, 3-6 years, 6-9 years, and 9-12 years as 47%, 28%, 14%, and 9% respectively.

Venugopal (2011) studied corneal injuries in the dogs and concluded that main cause of corneal ulceration in dogs was trauma. The incidence was highest in Pugs. It was observed that (79%) dogs were below one year of age and (14.9%) were below 6 months of age.

Ramani *et al* (2013) concluded that age group between 1-3 years (50%) showed a higher incidence of corneal ulcers followed by 4-7 years (29.2 %), above 8 years (20.8 %). Unilateral corneal ulcers were more common (75%). Higher incidence was recorded in the right eye.

Sale *et al* (2013) conducted a study on the incidence of ophthalmic affections in different age groups of below 1 year, 1-2 years, 2-5 years, 5-10 years and above 10 years. They observed that animals of all age groups showed the incidence of ocular affections.

Sex wise incidence of animals affected with corneal ulcers.

Turner and Blogg (1997) reported high incidence of corneal ulcers in males (80%) than females (20%) with a mean age of 6.5 years.

Ramani *et al* (2012) reported higher incidence of corneal ulcer in male dogs as compared to female dogs. The number of cases reported in male dogs (54.67%) is slightly higher than in female dogs (45.33%).

Ramani *et al* (2013) studied surgical bacteriology and grading of corneal ulcers in dogs and concluded that corneal ulcers occurred to an extent of 67% in male dogs and 33% of females.

Breed wise incidence of animals affected with corneal ulcers.

Holmberg (1981) studied the incidence of corneal erosions in dogs and reported highest incidence in Shih Tzu (73%) followed by Boxers (20%). Dogs below 6 months of age were more predisposed towards the development of corneal ulceration. The incidence was found more in males (80%).

Moore (2003) reported that boxer is the most common breed predisposed to develop indolent corneal ulceration. Incidence was found 24.56% in Boxers and other 45 different breed included mixed breed, poodles and poodle crosses, Golden retrievers, Corgis, Labrador retrievers, Springer spaniels and German Sheppard's and Sheppard crosses.

Stiles and Townsend (2007) concluded that corneal sequestrum is a common disorder in cats, particularly in the Persian and Himalayan breeds and it rarely seen in dogs and equine patients.

Kim *et al* (2009) observed high incidence of corneal ulcers in the Shih-Tzu (50%), Pekingese (25%), and Yorkshire terrier (16%) in the study of 32 dogs. The Maltese Terrier, Pomeranian, and Golden Retriever were at low risk.

Thomson (2007) reported that brachycephalic breeds with pushed-in faces, such as Shih Tzu, Bulldog, French bulldog, Lhasa Apso, Pekingese, and Pug were more prone ulcerative keratitis.

Ramani *et al* (2012) found highest incidence of corneal ulceration in Pug breed followed by Spitz, Non-descript, Boxers and Labrador retriever. Dogs between the age group of 3 months to 3 years had the highest incidence. The incidence of corneal ulceration was found more in males.

Ramani *et al* (2013) found higher incidence of corneal ulcers was in Pug (33.3%) followed by Labrador (16.6%), Spitz (12.5%), Doberman (8.3%), Mastiff (8.3%), Boxer (8.3%).

Dawson and Sanchez (2015) conducted prospective study on 100 dogs (199 eyes) for the incidence of corneal surface diseases. Out of 26 dogs that developed the corneal surface disease, seven were brachycephalic breeds that included: Two French Bulldogs, one English bulldog, one Pug, one Cavalier King Charles Spaniel, one Lhasa apso, and one Chihuahua.

Singh et al (2016) reported highest incidence of corneal ulcer in Neapolitan mastiff followed by pug and mongrel dogs.

2.6 Causative factors and clinical signs associated with corneal ulcers

Aetiology of corneal ulcers

Kern (1990) reported that ulcerative keratitis and deep stromal ulcers was more common in brachiocephalic breeds as a central and/or paracentral ulcers position. Subnormal corneal sensitivity, less blinking frequency, central thinning of the pre-corneal tear film and relatively lagophthalmos eye were possible causes of ulceration.

Grover *et al* (1998) concluded that corneal epithelial defects are a known consequence of the failure to apply topical lubrication during general anesthesia in human beings. The prevalence of corneal epithelial defects in humans following general anesthesia is reported to be up to 10% in unprotected eyes and as little as 0.17% in eyes protected with lubricants or eyelid taping.

Moore and Nasisse (1999) viewed that microtraumas to cornea, tear film disturbances, disorders of the immune system (immune-deficiencies, autoimmune diseases), bacterial infection and corneal surgical interventions were responsible for the development of ulcerative keratitis.

Andrew (2001) concluded that corneal sequestrum in cats has been associated with feline herpesvirus 1 and chronic corneal irritation by physical factors like as entropion or distichiasis. Corneal sequestrum appears as a brown to a black lesion of corneal necrosis of 1–2 mm in diameter to more than half of the cornea in size. It may be associated with intense corneal neovascularization.

Bentley *et al* (2001) concluded that an abnormality in the adhesion process between anterior corneal epithelium, basement membrane and stroma may be responsible for pathogenesis of indolent corneal ulceration. Further any abnormalities with hemidesmosomes, corneal stroma and corneal nerve innervations were also reported responsible for pathogenesis of indolent ulcer in dogs.

Gilger *et al* (2007) studied different aetiologies for corneal ulcer and categorised into congenital origin, infectious cause, allergic origin, trichiasis, distichiasis, ectopic cilia, entropion, trauma, foreign body in eye and lack of tears productions.

Kim *et al* (2009) reported that keratoconjunctivitis sicca (KCS) was the commonest cause of ulcerative keratitis (31%) followed by lagophthalmos (28%), bacterial infection (11%), nasal fold trichiasis (11%) and corneal trauma (8%).

Hvenegaard *et al* (2011) found that centrally located indolent ulcers were more common (40.14%) and often presented with discontinuation of the epithelium (34.51%), discontinuation of the epithelium with neovascularization (23.94%) and discontinuation of the epithelium and granuloma (11.97%). Decreased tear production is a known risk factor for corneal ulceration and erosion.

Dawson and Sanchez (2015) conducted a prospective study on 100 dogs (199 eyes) for prevalence of corneal surface diseases perceiving prophylactic topical lubrication under general anesthesia. Schirmer tear test 1 (STT-1), slit-lamp biomicroscopy, and fluorescein staining was performed before general anesthesia and 24 hours post general anesthesia. Pre existing ocular diseased dogs were excluded from the study. Incidence of corneal surface disease was compared between the different lubrication groups. Even under protection of topical lubrication, 0.50% eyes developed a superficial corneal ulcer and 18.60% eyes developed corneal erosion in patients during GA.

Park *et al* (2016) conducted a retrospective case-control study to evaluate the prevalence and risk factors for the development of corneal ulcers after non-ocular surgery performed under general anesthesia in dogs. Among 732 dogs, 14 dogs (1.9%) developed corneal ulcers after non-ocular surgery operated under general anesthesia. Long duration of surgery, application of fentanyl patch postoperatively, small skull dogs and dogs that received neurosurgery were considered risk factor for the development of corneal ulcers.

Clinical signs in the affected animals

Whitley (1991) found the most coomon clinical signs associated with keratitis were ocular hyperemia, pain, photophobia, blepharospasm, variable degree of corneal infiltration vascularisation, inflammation in the anterior chamber, corneal ulceration. He observed that corneal ulceration can affect any layer of corneal or all of them and was accompanied by purulent to mucopurulent ocular discharge.

Croix *et al* (2001) reported common clinical signs like blepharospasm, conjunctival hyperemia, superficial corneal neovascularization, superficial cellular infiltration adjacent to the ulcerated area in cats diagnosed with non-healing indolent ulcer

Soontornvipart *et al* (2003) observed clinical signs like pain, blepharospasm, protrusion of the nictitating membrane, epiphora and vascularization of cornea in 88 cases of dogs and cats having ocular affections.

Hollingsworth (2003) concluded that assessment of surgical repair of corneal injury depends upon the clinical signs associated with the injury, the length of the laceration, the location (peripheral cornea vs. central cornea), and whether the laceration was full thickness or partial thickness. Prolapse of uveal tissue through the laceration and presence of blood or fibrin clots in the anterior chamber was obvious indication of full-thickness insult.

Bussieres *et al* (2004) observed melting corneal ulcer, stromal abscesses, and corneal perforations in equine patients. Among the three equine patients, two equine patients showed corneal perforations and iris prolapse. Corneal foreign body and hypopyon were also noted in one equine patient.

Thomson (2007) reported that brachycephalic breeds have less sensitive corneas than other dog's breeds and may display minimal signs of ocular pain.

Bouhanna *et al* (2008) observed clinical signs like brown-pigmented ulcer with mild oedema, corneal vascularization, moderate blepharospasm and mild conjunctivitis in a 9-year old Shih Tzu diagnosed with corneal sequestrum. Menace reflex, palpebral reflex and pupillary light reflexe were found normal.

Hvenegaard *et al* (2011) reported clinical signs like blepharospasm (69.72%), red eyes (conjunctival hyperemia and/or congestion) (69.01%) and ocular discharge (64.79%) associated with indolent ulcers in boxer dogs. High incidence of indolent ulcers was found in right eye (54.23%) compared to left eye (45.77%). Most ulcers were unilateral (n=130) whereas bilateral ulcers were reported in only six eyes (n=6).

Thomas (2012) reported blepharospasm, severe uveitis, corneal oedema, exudates of red blood cells (hyphaema) and white blood cells (hypopyon) within the anterior chamber of eye of 9 year male pug suffering from descemetocle.

Sarangom *et al* (2012) reported iris prolapse in six-month-old male nondescript dog. Prolapsed iris was found entangled in perforated cornea at 10 O'clock position. Clinical signs associated with iris prolapse were epiphora, severe blepharospasm, and photophobia. Ophthalmic examination revealed distorted cornea with focal corneal edema and fibrin clot at the site of laceration, a shallow anterior chamber with hyphema, miosis, and dyscoria.

Singh *et al* (2016) observed most common clinical signs associated with corneal ulcers in dogs were lacrimation, blepharospasm and periocular swelling.

2.7 Diagnostic tests for corneal ulceration

Ophthalmic examination

Miller and Crenshaw (1988) noted that direct diffuse illumination could be used for assessing corneal clarity, contour and symmetry and contact between eyelid margin and cornea whereas localization and extent of the lesions could be determined by direct focal illumination.

Renwick (1996) stated that the basic requirements for ophthalmic examination were a bright focal source of light, a transilluminator and a means of magnification.

Ollivier (2003) stated that cornea should be examined for loss of transparency, Opacity, pigmentation, vascularisation, growths, lacerations, presence of foreign body, Changes of contour and ulceration. 2.5.2 Ocular reflexes

Moore (2003) described cornea is innervated by ophthalmic branch of the trigeminal nerve whose normal function is ascertained by using a cotton-tip applicator or wisp of cotton to elicit a blinking response after it is touched to the peripheral cornea.

Mitchell (2011) stated that since ophthalmic diseases such as blindness could have a neurological component, a full neurological examination was warranted.

Palpebral reflex

Moore (2001) described positive palpebral reflex as a blinking response, when the ophthalmic branch of trigeminal nerve was stimulated by touching the medial or lateral canthus.

Mitchell (2011) stated that it was important to observe the normal closure of the eyelids before eliciting palpebral reflex and a normal blink response assessment was required before judging the menace and dazzle responses.

Menace response

Martin (2001) explained that threatening, sudden movement near the eye produces a blinking reflex known as menace response. Air currents are avoided by presentation of fingers or by using a transparent shield so as not to elicit a false positive response and the contralateral eye should be closed to determine that it was not a binocular response to the stimulus.

Moore (2001) described the two components of menace response as visual status and eyelid function. A positive menace response required a clear optic medium, a functioning retina and intact optic and facial nerves.

Pupillary light reflex (PLR)

Felchle and Urbanz (2001) noted that PLR test effectively evaluated the function of retina, optic nerve and iris sphincter muscle.

Maggs (2008) observed that PLR was decreased or absent in case of iris atrophy, physical obstruction of the pupil, iris ischemia prior to use of a dilating drug or high concentrations of circulating epinephrine in case of fearful animals.

Mitchell (2011) opined that PLR was not a function of vision, as blind animals had a normal PLR as in case of cataracts or occipital cortex lesions and an absence of PLR could be noted in animals with normal vision as in conditions of iris atrophy.

Featherstone and Heinrich (2013) stated that a direct PLR was elicited by shining a bright focal light source at the eye which resulted in constriction of the ipsilateral pupil and indirect PLR was the simultaneous constriction of the contralateral pupil.

Cotton ball test (Tracking test)

Martin (2001) noted that the cotton balls dropped into the visual fields of the patient was a common means to evaluate vision. Initial impressions seen in the cotton ball test were important since animals got easily bored with the test.

Schirmer's Tear Test

Gelatt (1975) described Schirmer tear test (STT) as the most common test which is performed by placing a 5×35 mm of Whatman filter paper no.41 in a medioventral palpebral *cul-de-sac* of an unanaesthetized eye for one minute and recording the length of wetting of the strip.

Ludder and Heavner (1979) recorded decreased tear formation following administration of atropine topically or systemically, alone or in conjunction with general anaesthesia in dogs using STT strips.

Hollingsworth *et al* (1992) studied the effect of topically administered atropine on tear production in the eyes of 19 dogs. It was concluded that both eyes had a significant decrease in tear production. Effect was most marked up to 120 min after atropine instillation and then tear production returned to baseline values by 300 min after instillation.

Kaswan (1995) reported that STT values can be influenced by topical medications e.g. atropine decreases the tear production. Topical solutions may falsely increase tear production values whereas fear experienced by animal increases sympathetic stimulation and falsely decreases tear production values.

Berger and King (1998) studied fluctuation and variation in canine tear production from the results of daily Schirmer tear test (STT-1) without any topical anaesthetics and with topical anaesthesia (STT-2) weekly. It was reported that fluctuations in STT values occurred both daily and weekly. Fluctuations were only biologically significant on a week to week basis. There were significant differences between STT-1 and STT-2 values in dogs. Results also indicated that weight has a significant effect on STT values, with higher values measured in dogs having more body weight.

Herring *et al* (2000) described that general anesthesia (GA) and sedation protocols have been shown to reduce STT-1 readings significantly. STT-1 readings were significantly lower after 24 hours post-GA when compared to pre-GA readings. Although STT-1 readings have been reported to return to pre-GA values at 24 h postoperatively, but remained lower in this current study.

Bowersox and Criox (2001) reported that clinically normal animals may have STT values as low as 5 mm/ min, and suggested that the interpretation of the values should be done in light of clinical signs.

Kotani (2001) undertook studies on estimation of tear production rate by Schirmer tear test (STT) in dogs. STT values for adult canine were reported as 21.30±3.80 mm/min, 18.89±2.62 mm/min and 18.64±4.47 mm/min respectively. STT reading of 11–14 mm/min were considered moderately low and readings of equal to or less than 10 mm/min were considered low.

Lin and Wu (2002) performed Schirmer tear test on 50 dogs suffering from various corneal affections. On ophthalmic examination, bilateral involvement of eyes was observed in 30 dogs and unilateral involvement in 20 dogs. Bilateral ones tend to have more severe clinical lesions. Among 17 dogs affected bilaterally STT readings suggested that 10 eyes had mild or early KCS (STT 11-14 mm/ min), 4 eyes had moderate KCS (6-10 mm/ min) and 20 eyes with severe KCS (0-5mm/ min). Ten dogs with unilateral disease, only 6 eyes had moderate KCS (6-10 mm/ min) while 4 eyes showed severe KCS (0-5 mm/min).

Thangamuthu and Varshney (2002) conducted a study to generate baseline data on tear production in dogs and to see the effect of sex, age, body weight and breed on tear production. Overall STT values for left and right eyes were 22.54±0.41mm/ min and 22.62±0.41mm/ min respectively. Tear production pattern of right and left eyes was almost similar. Sex, age, body weight and breed did not significantly influence the STT values.

Hartley *et al* (2006) subjected different aged 100 dogs to STT every 2 hours during the day in the randomly chosen eye. It was observed that time of day and age of animal statistically significantly effected tear production. Mean STT values taken at 10.00 a.m. were 0.7 mm lower than the values taken at 4.00 p.m. (0.04mm). Mean STT decreased by 0.4 mm for every one year of age increased. It was concluded that tear production decreases with age in the normal dog and the greatest difference was between 10.00 a.m. and 4.00 p.m.

Anoop *et al* (2015) used Schirmer tears test (STT) for diagnosis of kerato-conjunctivitis sicca (KCS) and reported mean value of Schirmer tears test (STT) as 10.31±0.58 which was below normal range suggestive of kerato-conjunctivitis sicca (KCS).

Fluorescein dye test

Maurice (1967) stated that topical fluorescein can be used in veterinary ophthalmology to detect the corneal epithelial defects, to test nasolacrimal duct patency and to assist in the measurement of intraocular pressure by using Goldmann and Draeger tonometers.

Slatter (1990) used Fluorescein to detect corneal defects, conjunctival epithelial defects and pre corneal tear-film deficiencies. Hydrophilic fluorescein dye does not stain intact healthy corneal epithelium or to Descemet’s membrane. However when the corneal integrity lost, dye enters the water-soluble corneal stroma and stains the intracellular spaces only.

Strubbe and Gelatt (1999) described the use of the Seidel test to check the leaky cornea by applying fluorescein dye onto the corneal surface. The leaky cornea was detected by naked eye or with the aid of magnification.

Felchle and Urbanz (2001) reported that fluorescein dye will not penetrate the intact lipophilic corneal epithelium, but stain corneal defects only examined under the blue filter of the ophthalmoscope in a dark room.

Lin and Wu (2002) diagnosed 110 cases of ulcerative keratitis in 90 dogs and 20 cats. Diagnosis and assessment of ocular lesions were based on clinical signs, slit lamp biomicroscopy, fluorescein staining, STT, examination of the nasolacrimal system, and microbiological cultures.

Moore (2003) diagnosed chronic corneal epithelial defects or indolent corneal ulcerations from its classic appearance with the use of fluorescein stain which borders the epithelial lip.

Ollivier (2003) stressed the use of magnification system, fluorescein dye, corneal cytology and culture for diagnosis of corneal diseases especially ulcerative keratitis at an early stage of the disease in dogs and cats.

Singh *et al* (2016) reported that external ophthalmic stain (fluoresceine sodium) was a ideal diagnostic tool for detection of corneal ulcer and conjunctival defects. Fluorescein sodium stain was taken up by exposed corneal stroma and defining the margins the corneal ulcer green.

Corneal Microflora

Gerding and Kakoma (1990) isolated coagulase positive *Staphylococcus aureus* and *Staphylococcus intermedius* and coagulase negative *Staphylococcus epidermidis*, *Corynebacterium* sp. *Bacillus* sp, *E. coli*, *Serratia* sp., *Proteus* sp., *Citrobacter* from the canine conjunctival sac.

Moore and Nasisse (1999) concluded that *Staphylococcus* spp., *Streptococcus* spp. and *Enterococcus* spp. were the opportunistic occular bacteria that can make ocular infection more severe. They produced a number of enzymes and enterotoxin that enhance the progression of the infection and the destruction of the cornea.

Strubbe and Gelatt (1999) concluded that corneal swab cultures are more sensitive than corneal cytology for the prompt diagnosis, bacterial speciation and susceptibility testing for the successful management of bacterial corneal diseases.

Kaye *et al* (2003) studied corneal scraping and conjunctival swab culture for the incidence of bacterial keratitis in 61 human patients with mean age 56.5 years. Isolated micro flora includes *S. pneumonia*, coagulase negative staphylococci, Staphylococcus *aureus, Bacillus* spp, lactose fermenting Coliform (LFC), *Pseudomonas* sp, *Neisseria* sp, *Corynebacterium xerosis*, *Gemella morbillorum, Paecilomyces* sp., and a yeast. No viruses were isolated from the conjunctival swabs. Antibiotic sensitivity test revealed that two *S. pneumoniae*, *Bacillus* sp, and *G. morbillorum* were resistance to Ciprofloxacin but sensitive to teicoplanin.

Ollivier *et al* (2003) isolated Staphylococcus spp. (40%), Streptococcus spp. (25%), including β- Streptococcus spp. (16%) and α -Streptococcus spp.(9%), Escherichia coli (5%), Corynebacterium (4%) and Klebsiella (1%) from the conjunctival swab cultures from canine eyes.

Lassaline *et al* (2005) isolated the Pseudomonas spp. from the swab culture in three equine eyes affected with corneal ulceration and severe keratomalacia. Two eyes showed concurrent fungal infection.

Ledbetter and Scarlett (2008) isolated Clostridium, Peptostreptococcus, Actinomyces, Fusobacterium, and Bacteroides species from the corneal samples of domestic animals with ulcerative keratitis. Majority of these infectious bacteria were mixed anaerobic and aerobic bacteria. It was concluded that resident ocular pathogens and previous corneal abnormalities predispose the cornea to anaerobic bacterial infection.

Vongsakul *et al* (2009) cultured conjunctival swabs of 12 eyes revealed Staphylococcus spp. in 8 eyes, Streptococcus spp. in 2 eyes, Streptococcus spp. and Enterococcus spp. in 1 eye, and no bacterial growth in 1 eye.

Ramani *et al* (2013) isolated common bacterial pathogens from corneal swab culture from canine eyes includes Staphylococcus spp. (54%), followed by *Escherichia coli*. (17%), Bacillus spp.(8%).

Anoop *et al* (2015) study the 86 eyes of dogs suffering from varying grades of pigmentary keratitis and isolated the main microflora from the corneal culture of dogs were *Staphylococcus aureus* (40%), followed by *Enterococci* spp (16%), *Staphylococcus intermedius* (10%), *Corynebacterium* spp. (12%), *Klebsiella* spp. (7%), *Bacillus* spp.(4%), *E. coli* (4%) and *Actinobactor* spp. (6%) .

Corneal cytology

Gerding *et al* (1988) considered non cornified corneal epithelial cells (superficial and intermediate), lymphocytes and polymorphonuclear neutrophils as normal types of corneal cell. But in corneal bacterial infection polymorphonuclear neutrophils, keratin debris, mucus, and bacterial clumps were predominant cells.

Slatter (1990) described that corneal cytology was a diagnostic tool used either alone or in combination with culture techniques for diagnosis and assessment of treatment regimen for conditions like deep melting or progressive corneal ulcers, corneal or conjunctival abscesses, chronic or severe keratitis, and keratitis that not respond to therapy.

Ollivier (2003) stated that cotton or dacron swabs, cytobrushes, spatulas and blunt end of sterile scalpel blade were different cytology samples collecting instuments and techniques. Cotton and Dacron swabs provide least traumatic method of retrieving exfoliative samples, greater numbers of deeper cells from specific areas were provided by spatulas or blunt end of scalpel blade whereas cytobrush provides superior diagnostic samples compared with others techniques.

Balicki *et al* (2011) concluded that impression cytology was a simple and non-invasive method of sampling corneal and conjunctival epithelium cells, which allows monitoring of the ocular disease process in dogs.

Venancio *et al* (2012) evaluated and compared cytobrush exfoliation cytology from palpebral surface of both nictitating membrane and conjunctival. Palpebral surface of nictitating membrane was considered preferred site for collection of cytological sample, technically easy and greater facility to perform and least possibility of iatrogenic damage to eye.

Anoop *et al* (2015) studied impression cytology of canine corneas and observed the infiltration of neutrophils, degenerative changes, presence of necrotic debris and squamous epithelial cells in samples.

2.8 Surgical Management of corneal ulceration

Conjunctival grafting

Bussieres *et al* (2004) used bridge conjunctival flap over the small intestine submucosa (SIS) graft for the repair of corneal stromal abscesses in the right eye of a 16 years old female Quarter horse. Leakage of aqueous humor was observed 3.5 weeks after surgery. The bridge graft in this patient became very thick due to excessive granulation tissue formation. Visual outcome was confirmed by menace reflex.

Dice (1981) stated that direct blood supply to the wound provided by conjunctival flap was very beneficial as serum contains collagenase inhibitors, which counteract collagenase produced in necrotic and infected corneal tissues.

Helper (1981) found that application of conjunctival flaps raises the temperature of the cornea and thus facilitate healing by increasing corneal cellular metabolism.

Holmberg (1981) studied the use of conjunctival pedicle grafts for the treatment of corneal perforation in four horses resulted in the preservation of functional vision in two eyes and a third cosmetically acceptable eye. In the fourth horse which having severely damaged cornea and had an intense uveitis went on to develop a phthisis bulbi. He concluded that conjunctival pedicle grafts helped in healing of corneal ulcers by preservation of corneal integrity, minimizing lesions incompatible with functional vision and replacement of lost corneal tissue. Increased blood supply to the healing cornea was an added benefit not obtained by other procedures such as lamellar corneoscleral transposition or full thickness corneal graft.

Scagliotti (1988) used transconjunctival island graft for the treatment of deep corneal ulcers, descemetoceles, and corneal perforations in 35 dogs and 6 cats. Island grafts were circular plugs of conjunctival tissue harvested from the palpebral conjunctiva of the upper eyelid by using chalazion forceps, sutured directly into the corneal recipient bed.

Stadsvold (1995) studied different treatments for corneal ulcers in 61 eyes from 60 animals (53 dogs, 5 cats, 2 rabbits). The cornea of all eyes was debrided to remove loose epithelium. This was followed by gluing, cauterizing with 90% phenols, covering with a third eyelid or conjunctival flap or a combination of these methods or keratotomy. Keratotomy was most effective with complete healing in 100% of 27 treated eyes. Adequate anti-inflammatory therapy was however indicated when corneal epithelium was intact..

Laus *et al* (1999) used bilateral lamellar keratoplasty and conjunctival pedicle graft in dogs. Both the techniques were found satisfactory for the management of deep corneal ulcer. However, conjunctival grafting technique was found much easier and less time consuming than bilateral lamellar keratoplasty.

Hamor (2003) reported that third eyelid flap provided a readily available protective bandage for cornea in any condition where corneal coverage support or protection is required. Third eyelid may be sutured with upper eyelid or with superior bulbar conjunctiva particularly in brachycephalic breeds.

Hollingsworth (2003) suggested different surgical protocol for corneal surgery include corneal laceration suturing, conjunctival pedicle graft, transconjunctival island graft, advancement graft, total conjunctival graft, frozen tectonic cornea1 graft, porcine small intestine submucosa graft, and superficial keratectomy. Conjunctival pedicle graft technique was found much superior that the other techniques.

Soontornvipart *et al* (2003) performed conjunctival pedicle grafts in dogs and cats for the treatment of deep corneal ulcers with success rate high as 93.18%. Success rate did not significantly depend on breed, age, and sex of the patient, size and depth of the corneal lesion, selection of suture material and antibiotics, but significantly depends on surgical techniques and type of technique performed.

Maggs (2008) demonstrated that opacity resulting from uncomplicated stromal wounds can be limited by the supervised use of corticosteroids, provided, the infection has been controlled, and an epithelial covering as demonstrated by fluorescein has been established. Conjunctival flaps were indicated for repair of large defects for covering the recurrent corneal erosions and for the treatment of deep or progressive ulcers.

Kim *et al* (2009) studied different treatment protocols in dogs having corneal ulcers. They concluded that superficial corneal ulcers took average 5.1 to 13.4 days to heal treated with medication whereas deep corneal ulcers, treated with conjunctival flap construction, took 28.4 to 40 days to heal. Conjunctival flap construction was an effective treatment for deep corneal ulcers. Success rate was achieved 100% in superficial corneal ulcers and 55% in deep corneal ulcers respectively.

Jhanji *et al* (2011) reported that corneal perforation resulted from variety of infectious and non-infectious disorders required prompt management. Small perforations respond reasonably well to corneal gluing techniques while peripheral perforations can be best managed with a partial conjunctival flap and keratoplasty.

Ortiz *et al* (2011) performed superficial keratectomy and 360° conjunctival flap for bullous keratopathy in a two-year-old male Pinscher dog. 360º conjunctival flaps were maintained for 60 days. After 120 days 2nd superficial keratectomy was done to restore the corneal transparency and third eyelid flap grafting was made. 30

days post 2nd superficial keratectomy, third eyelid flap was removed. Corneal pigmentation of tempo-nasal quadrant and conjunctivalization in inferior nasal quadrant was observed. The axial portion of the cornea was transparent and vision was restored.

Grad (2012) performed conjunctival graft in a Pug having descemetocele. Extended care was needed post-operatively to achieve good results. It was concluded that conjunctival grafts was a good surgical technique for treatment of deep corneal ulcers and descemetocele.

Amniotic membrane grafting

Barros *et al* (1998) evaluated the use of glycerol preserved equine amniotic membrane as replacement graft for full-thickness corneal defects in eighteen mixed-breed dogs. They concluded that xenologous amniotic membrane can be useful as a tectonic graft in the repair of full-thickness lesions of the cornea of dogs.

Fukuda *et al* (1999) explained that anatomically amniotic membrane was avascular in nature made up of single layer of metabolically active cuboidal to columnar epithelium firmly attached to a basement membrane.

Kruse *et al* (1999) used cryopreserved multilayer human amniotic membrane transplantation in eleven human patients with deep corneal ulcers. Following transplantation epithelial healing and amniotic membrane dissolution was observed 4 weeks and 12 weeks respectively. Multilayer amniotic membrane transplantation was useful technique for deep corneal ulcers and descemetocele as it allow corneal surface reconstruction within short time periods.

Rodrigues-Ares *et al* (2004) used multilayer amniotic membrane transplantation (AMT) in fifteen eyes with corneal perforations of different sizes. Two to four layers of amniotic membrane were made and sutured at corneal recipient bed. Mean epithelialization time was 3.7 weeks and success rate 73% (11/15 eyes) was observed. It is concluded that multilayer AMT is effective for treating smaller diameter (1.5 mm) corneal perforations. Multilayer amniotic membrane transplantation (AMT) could be advocated as a good alternative to penetrating keratoplasty. Increasing no. layers of amniotic membranes significantly increased the epithelialization time of the perforations.

Tourino *et al* (2004) observed that when stromal side of amniotic membrane (AM) faces cornea, then AM acts as a graft to facilitate epithelial cell migration, reinforces adhesion of the basal epithelium, promotes cellular differentiation and prevents apoptosis.

Gomes *et al* (2005) studied biochemical nature of amniotic membrane and described that amniotic membrane is a biologic tissue that can be used as a graft for corneal and conjunctival reconstruction in ocular surface diseases. It is avascular and possesses antiangiogenetic, anti-scarring and anti-inflammatory properties. They were of viewed that amniotic membrane should be used as a substrate upon which cells can migrate, regenerate, forming new and healthy tissue but not as a substitute for other ocular reconstruction techniques.

Lassaline *et al* (2005) used equine amniotic membrane (EAM) in three horses with corneal ulceration and severe keratomalacia without any conjunctival graft and concluded that equine AM can be used successfully to preserve both globe structure and limited vision, as well as to optimize cosmetic appearance of eye in corneal ulceration and severe keratomalacia like conditions.

Ollivier *et al* (2006) subjectively compared outcome of amniotic membrane transplantation (n=9 eyes) and bulbar conjunctival grafting following keratectomy in equines after excision of corneo-limbal squamous cell carcinoma (SCC). Strontium-90 irradiation or cryotherapy was also used in 3 eyes. They concluded that amniotic membrane transplantation (n=9 eyes) received eyes showed a minimal level of scarring in a cornea and regained a greater transparency in comparison to bulbar conjunctival graft treated eyes.

Kalpravidh *et al* (2009) used canine amniotic membrane (CAM) along with a third eyelid flap after removal of large dermoids in seven eyes. Corneal epithelialization was attained within 2 weeks after the transplantation without any neovascularization and scarring of cornea. Five eyes attained normal transparency of cornea within 5 weeks. It was concluded that canine AM transplantation can promote corneal healing after the excision of large dermoids in dogs.

Kim *et al* (2009) evaluated the effectiveness of bovine freeze-dried amniotic membrane (FD-AM) for surgical treatment of corneal ulceration in dogs. Epithelial healing was significantly increased in AMT group. Greatest healing rate and epithelial cell proliferation was achieved with AMT compared to the other treatment regimes.

Wichayacoop *et al* (2009) concluded that topical application of supernatant from human amniotic epithelial cell culture (HAEC) alleviated inflammation in induced corneal ulcer of dogs, possibly via inhibition of interleukin-1beta (IL-1beta) and nitric oxide (NO) production.

Vongsakul *et al* (2009) compared canine amniotic membrane (AM) transplantation in conjunction with a third eyelid flap and third eyelid flap alone to promote healing of created deep corneal ulcers. The average time to complete corneal epithelialization in the eyes receiving the AM transplantation in conjunction with the third eyelid flap was 7.33±0.21 days which was significantly shorter than the average time of 9.17±0.31 days observed in the eyes receiving only the third eyelid flap. Canine AM provides rapid epithelialization without neovascularlization and scarring of the cornea which is paramount for healing of cornea.

Cirman *et al* (2014) described that amniotic membrane was the innermost layer of the placenta. It acts as scaffold for the regeneration of new cells and promotes epithelialization. It has antimicrobial properties and decreases inflammation, fibrosis and neovascularization. It can be used for the reconstruction of conjunctival and corneal defects and for the treatment of corneal ulcers. They also proposed that amnion derived cells stem cell like characteristics and contributed to cell based treatment of ocular surface diseases

Agraval *et al* (2017) used frozen fresh amniotic membrane after excision of conjunctival neoplastic lesions in 35 males and 18 females with mean age 54.9 (range 19–88). Most common conjunctival neoplasia was invasive malignant melanoma confirmed by histopathology. Success outcome without any complications was observed in 77.3% of patients. Complications in terms of minimal scarring (11.3%), symblepharon (11.3%), and granuloma (7.5%) were noticed. It was concluded that use of fresh frozen amniotic membrane graft (AMG) can improve the local surgical outcome by improving healing and reducing scarring.

Others biomaterial grafts

Hacker (1991) concluded that frozen corneal grafts was a good tool to provide support to deep corneal ulcers or keratectomy sites, thereby preventing corneal rupture in dogs and cats. These grafts require harvesting of donor corneal tissue, stored in a bottle of neomycin-polymyxin B-gramicidin ophthalmic solution and need to suture at recipient corneal bed.

Voytik *et al* (1997) described anatomical structure of porcine small intestine submucosa (SIS) graft derived from jejunum. All mesenteric tissues, tunica mucosa, serosa and tunica muscularis were mechanically removed from jejunum using an abrasion technique. Mechanical debridement and washing with hypotonic solutions lyzed the few remaining resident endothelial cells and fibrocytes leaving a sheet of collagen which was used as SIS graft. It was rich in collagen (type I, III and VI), glycosaminoglycans (hyaluronic acid, chondroitin sulfate A and B, heparin, and heparan sulfate), proteoglycans, and glycoproteins (fibronectin), which were known to have important roles in tissue repair. Fibroblast growth factor and transforming growth factor β have been identified in SIS, influence and modulates wound healing and tissue remodeling.

Hansen and Guandalini (1999) used frozen corneal lamellar grafts and nictating membrane flaps in dogs and cats to repair deep corneal ulcers. Frozen lamellar corneal graft was found safe for restoration of the optical function of cornea.

Featherstone *et al* (2001) used commercially available porcine small intestine submucosa (SIS) as corneal graft material to provide support for deep ulcers or keratectomy sites in ten cases of the feline corneal disease. The SIS graft was prepared by cutting it into the desired size and shape and rehydrated. Graft should be about 1.0 mm larger than the corneal defect and sutured to recipient corneal bed. Vision restoration was achieved in eight cats. One cat underwent enucleation while second cat had aqueous humor leakage. It was concluded that SIS graft should be used with caution in the presence of an infectious component as it does not bring a vascular supply to the corneal lesion.

Bussieres *et al* (2004) found that SIS graft was an inexpensive, easy-to-handle, good alternative graft biomaterial, suitable for repair of full-thickness corneal wounds. Corneal perforations and full-thickness lacerations were repaired with SIS in five dogs, two cats and four horses followed by conjunctival grafting. Visual outcome was 93% in the study. It was noticed that conjunctival grafting over SIS graft provided more structural support and brought a readily blood supply to compromised cornea. It also offered significant antimicrobial and anti collagenase activities and help to seal the corneal graft defect by bringing in fibroblasts and collagen network.

Jhanji *et al* (2011) observed that corneal perforation may be associated with prolapse of ocular tissue that requires prompt diagnosis and treatment. Although infectious keratitis is an important cause, corneal xerosis and collagen vascular diseases should be considered in the differential diagnosis, especially in cases that do not respond to conventional medical therapy. Moreover medical therapy is a useful adjunct to the surgical approach for most of the corneal perforations. Depending on the size and location of corneal perforation, treatment options include gluing, amniotic membrane transplantation and corneal transplantation.

Dulaurent *et al* (2014) evaluated the efficacy of bovine pericardium graft for the treatment of deep melting corneal ulcers in three dogs and corneal sequestra in three cats. Bovine pericardium graft was sutured to the recipient cornea following keratectomy. Two months after surgery five corneas out of six (two in dogs and three in cats) had healed with focal corneal scarring. One dog which had progression of the keratomalacia, became blind. It was concluded that bovine pericardium graft offered a promising option for surgical reconstruction of the cornea following keratectomy for the management of corneal ulcers and sequestra.

Dorbandt *et al* (2015) compared the success rate of conjunctival pedicle flap (CPF) alone and combination of CPF with acellular submucosa implant for the repair of deep or perforating corneal wounds in dogs. Combined success rate of all corneal wounds was 93%, whereas for corneal perforations, descemetoceles, and deep stromal wounds was 89%, 95%, and 100%, respectively. There was no difference in overall success rate between groups. A comparable success rate was achieved for deep or perforating corneal wounds stabilized with a CPF alone vs. a CPF plus acellular submucosa.

Surgical debridement

Mauger (1994) observed that disodium salt of 2.6% EDTA as chemical debridement or keratectomy dissolved calcified depositions within the corneal stroma. EDTA solution soaked cotton-tipped applicator was used to gently massage the calcified lesions. Depending on the thickness and extent of the calcified lesions, massage application took 45 to 60 minutes to remove the calcium deposits. The mineralized stroma tends to flake off and the areas of corneal degeneration became ulcerated and later treated as uncomplicated ulcer.

Turner and Blogg (1997) studied the effect of multiple striate keratotomies: a treatment for corned erosions caused epithelial basement membrane disease. They were of view that multiple striate keratotomy was safe, effective and well-tolerated technique for the treatment of persistent corneal erosions thought to be caused by corneal epithelial basement membrane disease.

Stanley *et al* (1998) performed superficial keratectomy, grid keratotomy, and debridement by sterile cotton swab and were of view that superficial keratectomy and grid keratotomy were highly successful techniques for the treatment of persistent corneal ulcers

Whitley and Gilger (1999) studied the use of tincture of iodine, dilute povidone-iodine, trichloracetic acid, and phenol for chemical debridement of necrotic cornea. Iodine was most commonly used chemical debridement agent and was theorized to alter the anterior stromal surface, allowing for epithelial adhesion.

Croix (2001) compared mean healing times after debridement with grid keratotomy and superficial keratectomy in cats with non-healing ulcers and concluded that brachycephalic cats appear to be more predisposed to the development of non-healing corneal ulcers. It was concluded that superficial debridement and grid keratotomy decreased the mean healing time of non-healing ulcers.

Bentley (2005) studied the occurrence of spontaneous chronic epithelial defects (SCCEDs), in middle-aged dogs. On histopathology, the loss of corneal epithelial basement membrane and formation of a superficial acellular hyalinized zone in the stroma was found. Epithelial debridement, anterior stromal puncture, grid keratotomy, and superficial keratotomy were treatment options for spontaneous chronic epithelial defects (SCCEDs) and offers significant success rate.

Cullen and Grahn (2005) performed lamellar keratectomy and conjunctival graft in a female Shis Tzu dog having a corneal foreign body. Lamellar keratectomy followed by conjunctival graft was found ideal surgical technique which promotes corneal healing within a short time period.

Janssens (2007) performed punctate keratotomy, grid keratotomy, superficial keratectomy and debridement techniques in dogs having indolent corneal ulcers. Grid keratotomy and superficial keratectomy were better surgical techniques which promote rapid healing of ulcers within a shorter period of time. Main disadvantage with punctate keratotomy was a greater risk of deeper damage to the cornea. Success rate was slightly lower than linear keratotomy, perhaps as a result of the reduced stromal surface area exposed with punctate wounds as compared with linear.

Jones *et al* (2007) performed grid keratotomy on the eye of a llama. Technique involved incision of the cornea which extended into the healthy corneal stroma to encourage adherence between the epithelium and stroma. Combined use of epithelial debridement and grid keratotomy significantly decreased healing times for indolent ulcers.

Asghari and Gharachorou (2011) studied the role of antibiotic administration for the treatment of corneal ulcers in dogs. In the study 54% of the dogs were cured with antibiotic administration, 28% needed corneal surgery and 18% of the dogs underwent evisceration due to perforation of the cornea. It was concluded that most of the superficial bacterial corneal ulcers can be treated with proper administration of the broad-spectrum antibiotics while deep corneal ulcers need corneal surgerical procedure like grid keratotomy and superficial keratectomy.

Hvenegaard *et al* (2011) noticed that indolent ulcers were superficial corneal ulcers secondary to several changes on the corneal surface, frequently observed in middle-aged Boxer dogs. They results in acute onset of pain and required appropriate treatment. Debridement, cauterization, administration of proteinase inhibitor eye drops, prophylactic topical antibiotics, and oral vitamin C were considered effective clinical management for indolent ulcers. It was concluded that debridement and cauterization were fundamental to corneal healing, since it was capable to heal 100% of the wounds, most of them in less than 30 days.

Sarbani and Himangshu (2011) performed grid keratotomy for the treatment of atypical indolent corneal ulcers in a Boxer dog. They considered grid keratotomy safe and effective technique for the treatment of superficial indolent ulcers refractory to topical antibiotic treatment.

Singh *et al* (2014) compared different surgical techniques for the management of corneal ulcers in dogs and concluded grid keratotomy and third eyelid flap techniques effective in surgical management with minimal complications in both superficial and deep ulcers as compared to punctate and conservative treatment.

2.9 Complications associated with corneal ulcers

Featherstone *et al* (2001) reported complications in ten cases of feline corneas where SIS grafts were applied. One cat underwent enucleation due to the progression of keratomalacia and a second cat acquired the aqueous humor leakage which required enhancement procedure (conjunctival flap), to seal the cornea. Anterior synechia was believed to occur secondary to aqueous humor leakage and pre or post surgical uveitis.

Bussieres *et al* (2004) observed the most common complications following corneal surgery were aqueous leakage, conjunctival flap dehiscence, synechia, cataract and fibrin in the anterior chamber. Fundus examination showed signs of vitreal degeneration. Retinal scarring were multiple linear streaks possibly thought to be from a previous retinal detachment. Panuveitis was suspected as causes of these lesions because the retina was normal examined before surgery.

Sarangom *et al* (2012) observed uveitis, endophthalmitis, panophthalmitis, anterior synechia, pigmentation panophthalmitis, anterior synechia, pigmentation panophthalmitis, anterior synechia, pigmentation like complications associated with iris prolapse and corneal laceration.

Dorbandt *et al* (2015) noticed corneal fibrosis at the site of injury, corneal vascularization, corneal pigmentation, and recurrent corneal ulceration like important complications postoperatively. However, none of thesecomplications significantly affected visual outcome or success rate. Glaucoma was not observed post-surgical complication in the study.

CHAPTER III

MATERIALS AND METHODS

Present study was conducted on 28 eyes of 27 dogs presented with the history and clinical symptoms of ulcerative keratitis or corneal ulcers to the Department of Veterinary Surgery and Radiology, GADVASU, Ludhiana from the period of March 2016 to May 2017

3.1 Brief outline of the study

Signalment and detailed anamnesis from each case presented for ocular ailments were taken. Information regarding previous disease conditions and treatment regimen pertaining to ocular ailments was collected. Each animal underwent detailed physical examination and specific ophthalmic tests on the day of presentation. Blood samples were collected from cephalic vein for haematological examination prior to surgery. Conjunctival or corneal swab samples and corneal impression smears were collected for microbiology and corneal cytology work respectively. Presented animals were divided into following three groups (Table 1) based on the surgical technique adopted: **Group I (n=13)** comprised of bulbar conjunctival grafting, **Group II (n=4)** comprised of Amniotic membrane grafting and **Group III (n=11)** comprised of surgical debridement. Group I was divided into two sub-groups i.e. Group I A (n=7) in which conjunctival pedicle grafting was performed and Group I B (n=6) in which conjunctival bridge grafting was performed. Group III (n=11) was sub divided into two groups i.e. Group III A (n=6) in which debridement with beaver blade no.# 64 was done and Group III B (n=5) in which debridement with sterile cotton swab was done.

Table 1: Group wise distribution of corneal ulcers on the basis of surgical techniques used

Groups	Subgroups	No. of eyes	Surgical procedure used
I	Group I(A)	7	Bulbar Conjunctival pedicle graft
	Group I(B)	6	Bulbar Conjunctival bridge graft
II	-----	4	Human Amniotic Membrane overlay Patch
III	Group I(A)	6	Debridement with beaver blade no.# 64
	Group I(B)	5	Debridement with sterile cotton swab

3.1.1 Signalment

Breed, age, sex and history regarding ocular conditions and type of lesion, duration of lesions, symptoms of lesion, previous medications administered and previous health related ailments were recorded.

3.1.2 Ophthalmic Examination

Pre-operatively, all animals were subjected to detailed ophthalmic examination using following tests**:**

Examination of eye

A thorough evaluation of eye for confirmatory diagnosis of corneal ulcers was made by complete ophthalmic examination included Fluorescein dye test by using Fluorescein dye strips (Optechnics unlimited, DLF city, Gurgaon, India), Schirmer's tear test by using Schirmer's tear strips (Micromed International Ahmedabad, India). Topical local anaesthetic proparacaine 0.5% (Paracain®: Sunways India Pvt. Ltd. Mumbai, India) was used to anaesthetise the eye.

Visual function tests/ocular reflexes: Visual activity of the eye was assessed by standard eye procedures as follow (Ofri 2008).

Palpebral reflex: Palpebral reflex was performed by tapping the lateral and medial canthus of eye to elicit a blink response. It determines the integrity of maxillary branch of fifth cranial nerve (trigeminal nerve) and auriculpalpebral branch of seventh cranial nerve (facial nerve).

*Menace Reflex***:** Normal menace reflex was elicited as closure of eyelids when examiner stimulated the eye in a way that tried to threaten the animal usually by waving the hand in front of eye of animal and meantime avoiding the inflow of outer air current into eye.

Pupillary light Reflex (PLR): A pen focal light source (Pen torch) or bright light source was projected directly into eye. Resultant pupillary constriction was observed with naked eye by examiner as pupillary light reflex.

Cotton ball test (Tracking reflex): CBT was performed by dropping a small cotton ball on either side about 20-30 cm in front of each visual field. Reaction of the dog toward object was recorded.

Special Diagnostic Procedures (Moore 2003): Specific diagnostic procedures like Fluorescein dye test, Schirmer's tear test, culture sensitivity test and corneal impression cytology were performed on each animal presented for corneal ulcer.

Fluorescein Dye Test

Fluorescein dye test was performed by inoculating the commercial available Fluorescein dye strips into affected eye. These strips were impregnated with special fluorescein dye. Dye stains only the corneal defect whenever, pre corneal tear film disruption was present. These strips when moistened with sterile saline solution or directly inoculated into eye, stained any epithelial defect in cornea**.** Location of ulcer was marked by dividing the cornea into four quadrants. Any defect in any of the quadrants was demarcated as dorso nasal, ventro nasal, dorso temporal and ventro temporal ulcer. Positive dye test was interpreted as green staining of lips or margins of epithelial defect. Excess of dye was drained out from nostrils through superior and inferior lacrimal puncta of eyelds.

Schirmer's Tear Test

Schirmer's tear test (STT) was performed to measure basal and reflex tear production and dry eye condition. STT was performed by using sterile, absorbent Whatman No.41 filter paper (5mm×40mm) (Micromed International Ahmedabad, India). The tip of Schirmer's tear strips were slightly bended and placed into the lower conjucntival Cal- de- sac for one minute. Strip was carefully taken out from conjunctival sac. Wetting of strip was noted and measured in millimetres scale printed on the strip (Gellat, 1991; Morreale, 2003). Strip was printed from 1-25 mm reading. Wetting of strip was measured as tear production value accordingly. Based on the STT value, tear production of eye and health condition of eye was graded.

Culture and isolation of conjunctival sac and corneal micro flora

Corneal swab samples were taken using sterile cotton swab, before instillation of any medication. Sterile cotton swabs were opened near eye and moistened with sterile saline solution. Moistened cotton swabs were carefully rolled over the ulcer area and in the conjunctival sac and packed in the container available with cotton swab. Samples collected were transported to the microbiology laboratory and processed on the same day. These samples were inoculated in nutrient broth for bacterial culture on the day of sample collection and incubated aerobically at $37^{o}C$ for

24 hours and observed for bacterial growth. Bacterial culture from the nutrient broth was taken and inoculated on Brain-heart infusion agar (BHI) or Nutrient agar (NA) plates using quadrant–streaking methods. Petri plates were incubated aerobically at 37^{o}C for 24 hours. Bacterial colonies were studied for colony morphology, shape, size. Isolated individual colony was subjected to gram staining for bacterial identification (Quinn *et al* 1999).

Corneal impression smear cytology

Impression smear of cornea were prepared for examination and evaluation of exfoliative cytology of cornea. Impression smear was prepared by touching the clean glass slides directly to cornea especially the lesion area i.e. corneal ulcer. Impression smear was prepared from both eyes for comparative evaluation. Prepared smears were dried, stained with Leishman's stain (Jain 1986) and examined under oil immersion lens for predominant type of leukocyte cell, type of epithelial cells and bacterial clumps or fungal hyphae.

Culture sensitivity test (CST)

Brain-heart infusion agar was used for culture sensitivity test. Isolated bacterial colony was taken and inoculated in nutrient broth and incubated aerobically at 37^{o}C for 24 hours. Bacterial culture was taken from broth and swabbed on BHI plate and standard antibiotic discs (Himedia) viz. amikacin, amoxicillin, ampicillin, ciprofloxacin, gentamicin, kanamycin, neomycin, enrofloxacin, tobramycin, gattifloxacin, sulphathiazine, cephataxime, clindamycin, and tetracycline were applied on plate. Petri plates were incubated aerobically at 37^{o}C for 12 hours. Results were noted as per the size of zone of inhibition around the individual antibiotic disc and compared with the interpretive chart provided by the manufacturer and interpreted in terms of antibiotic sensitivity or resistance.

Haematology

Blood samples were collected from cephalic vein, preoperatively for every case in the ethylene diamine tetra acetic acid containg vials and processed on the same day. Whole blood sample (2 ml) collected in EDTA coated vials (Accuvete-Plus, Quantum Biological Pvt. Ltd) was used for determination of Haemoglobin concentration (Hb gm.%), Total leukocyte count (TLC), Platelets count, Packed cell volumes (PCV) parameters using Fully Automated Haematology Analyser (ADVIA

2120 Haematology system, Siemens Healthcare Diagnostics Inc., USA). Differential leukocyte count (DLC) was counted manually using Leishman stained peripheral blood smears. Blood smears were also evaluated for RBC, WBC, platelet abnormalities and other morphological changes

Instruments Used During Surgical Procedure

Various types of instruments used during surgical procedure for corneal ulceration included: Coaxial operating microscope (Shin Nippon OP-2, Ohira Co. Ltd-Japan), Eye drape, Eye speculum, Steven's tenotomy scissor, corneal scissor, Tying forceps, Mosquito forceps and Beaver blade.

Pre-operative procedures

Pre-operative medicinal therapy

Pre-operatively, all animals diagnosed with corneal ulcers were instilled topical intra ocular medicinal therapy from the day of presentation with 0.3% tobramycin topical antibiotics drops, topical non-steroidal anti-inflammatory drugs (NSAID) ketorolac, and topical artificial tear drops carboxy methyl cellulose sodium. Depending upon the severity of ulcer deep ulcers and descemetocele, parenteral antibiotics and non-steroidal anti-inflammatory drugs (NSAID) were also given.

Surgical procedure

All presented animals with corneal ulcers scheduled for corneal surgery received 0.3% tobramycin antibiotics eye drops for ocular asepsis and diluted solution of 0.5% povidine Iodine for extra ocular asepsis with frequency of two to three times 15 minutes apart prior to surgery. Injection of Cefotaxime @ 25 mg/kg body weight (Taxim® Alken Laboratories, Mumbai, India) was given to eliminate the systemic infection.

Anaesthesia

Anaesthetic protocol for conjunctival grafting and amniotic membrane grafting included premedication with combination of Gycoprrolate @ 0.01mg/kg (Pyrolate® Neon Laboratories Ltd, Mumbai, India), Acepromazine maleate @ 0.05mg/kg (Ilium- Acepril-10® Troy Laboratories Pvt. Ltd, Australia) and Butorphenol @ 0.2mg/kg body weight (Butodol® Neon Laboratories Ltd, Mumbai, India) by intramuscular route. Induction was performed with Diazepam @ 0.25 mg/kg of body weight (Lori® Neon Laboratories Ltd, Mumbai, India) and Ketamine @ 5 mg/kg of body weight (Aneket® Neon Laboratories Ltd, Mumbai, India) given by intravenous route “to effect” up to endotracheal intubation. Anaesthesia was maintained with 1 to 2% Isoflurane (Forane® Aesica Queenborrough Ltd, UK). Acepromazine was excluded from the premedication regimen of Pug breed dogs. Topical 0.5% proparacaine (Paracain® Sunways India Pvt. Ltd. Mumbai, India) local anaesthesia was used in animals subjected to surgical debridement.

Patient positioning and preparation

Patients were placed in lateral recumbency with affected eye on dorsal position. Soft bedding material was kept under the cranium of dogs to elevate and position the head and to make sure that nose was straight up slightly away from the eye intended for surgery. Light coloured commercially available eye drape with operating window in the centre having dimensions about 5×5cm was used for draping the eye during surgery. Rest of body was covered with sterile drape to achieve proper asepsis during procedure. Surgery was conducted by surgeon sitting on the lateral side of patient. Self-retaining adjustable ophthalmic eye retractor was used to held the eyelids in position.

Operating procedures

Bulbar conjunctival pedicle grafting **(Gellat 2011)**

Seven eyes of six animals were chosen for conjunctival pedicle grafting from the bulbar conjunctiva. Affected eye was secured with application of eye retractor. Affected eye was retracted out from the bony orbit just sufficient to expose the bulbar conjunctiva. Recipient ulcer margins were prepared for graft application through debridement of necrotic tissue and infected stroma. Depending upon the location of ulcer, grafts were harvested from superior, inferior or lateral bulbar conjunctiva whichever, was near to the ulcer to allow the graft to travel shortest distance. Once the ulcer location was determined, incision of sufficient length was made on bulbar conjunctiva parallel to limbus at distance of 1-2 mm to limbus. Second incision of same length about 1-2 mm wider than the size of ulcer was made parallel to the initial conjunctival incision and undermined by blunt dissection using Steven’s tenotomy scissors. Third incision was given at the base of either side of graft whichever, was easy to translocate or rotate the graft to the recipient ulcer site. Strip of conjunctiva thus created was extended up to the ulcer margins to cover the ulcer area. Conjunctival flap was sutured to cornea with simple interrupted suture pattern using 6-0 Polyglactin 910 absorbable suture material. Following conjunctival flap suturing, eyelids were closed by temporary partial tarsorrhaphy using non absorbable suture material and were kept for 10 days. After 10 days, tarsorrhaphy suture was removed and eye was examined for surgical outcome.

Bulbar conjunctival bridge/bipedicle grafting **(Gellat 2011)**

Six eyes of six animals were chosen for conjunctival bridge grafting from the bulbar conjunctiva. Affected eye was first secured with application of eye retractor and then eye was retracted out from the bony orbit just sufficient to expose the bulbar conjunctiva. Recipient ulcer margins were prepared for graft application through debridement of necrotic tissue and infected stroma. First incision was given on bulbar conjunctiva just 1-2 mm from the limbus. Incision was given parallel to the limbus and was extended to 180^{o} of conjunctiva. Second incision was given parallel to initial conjunctival incision. Conjunctiva was extensively undermined in between two parallel incisions. A bridge was created. This bridge flap was then advanced over the ulcer area and was sutured to cornea with interrupted suture pattern using 6-0 Polyglactin 910 absorbable suture material followed by temporary partially tarsorrhaphy using non absorbable suture material (Nylon) for 10 days. After 10 days, tarsorrhaphy suture was removed and eye was examined for surgical outcome.

Dry human amniotic membrane grafting

Dry human amniotic membrane grafting was performed in four eyes of 4 dogs as an on-lay patch. Commercially available ready to use human amniotic membrane was used. Following sterile draping, eye retractor was applied to expose the surgical site. Eye was retracted out of bony orbit either through gentle digital pressure or by medial canthotomy. Loose epithelium from lesion area was debrided either by sterile cotton swab or surgical blade. Dry amniotic membrane was cut into desired size 2-3mm wider than the ulcer dimension. Amniotic membrane graft havind two sides i.e. epithelial and stromal side. Stromal side of amniotic membrane was applied to epithelial surface of canine cornea for acting as graft. Two layered amniotic membrane graft was transplanted onto the corneal ulcer. Graft was applied to cover the whole corneal defect. Dry amniotic membrane was moistened by instilled few drops of sterile saline solution to enhance adherence of graft to cornea. Once amniotic membrane graft adhered to cornea, eye was closed by temporary partial tarsorrhaphy with nylon to protect the eye from self-trauma. Tarsorrhaphy sutures were removed after 10 days and eye was examined for surgical outcome.

Debridement

Superficial corneal ulcers were debrided using beaver blade no.# 64 (n=6) and sterile dry cotton swabs (n=5). Topical local anaesthetic drops of 0.5% propairacaine were instilled into affected eye to anaesthetise the cornea. Animals were kept in lateral recumbency. Sterile eye drapes were used to cover the eye. Necrotic corneal epithelium was debrided by Beaver blade no.# 64. Beaver blade was held at an angle of 45^o to corneal ulcer. Ulcer margin was debrided to freshen the epithelial margins. In group III-B dry sterile cotton swab was carefully rolled in circular motion over ulcer to debride the loose corneal epithelium. Two to three applications of cotton swab were made to refreshen the ulcer margins. No tarsorrhaphy was performed.

Temporary tarsorrhaphy

Temporary partial tarsorrhaphy was done in all animals subjected to conjunctival and amniotic membrane grafting, except in animals subjected to debridement group III. One or two horizontal mattress sutures were applied using 2-0 nylon non absorbable suture material. Needle penetration was started over the upper eyelid at the level of meibomian gland to exit at lower eyelid at same level. Horizontal mattress suture was applied keeping the knot on upper eyelid to prevent soiling of suture with ocular discharge. Knot was first tied with surgeon’s knot and subsequently finished in a “bow-tie” fashion to loosen the tarsorrhaphy suture for evaluation of cornea. Small opening near the medial canthus was left for topical medication. Tarsorrhaphy sutures in all animals were removed after 10 days and eyes were evaluated for surgical outcome.

Post-operative Care

Post-operative regimen in all groups includes parenteral antibiotics i.e. cefotaxim @ 25mg/kg body weight and NSAID i.e. meloxicam @ 0.2mg/kg body weight by intra muscular route for 5 and 3 days respectively. Topical NSAID ketorolac, antibiotics eye drops tobramycin, and artificial tear drops carboxy methyl cellulose sodium were advised to instilled 4 times daily for 4 days, 15 days and 1 month respectively.

Surgical outcome

A successful surgical outcome was defined in terms of acceptance of graft, restoration of normal visual activity, minimum to absence of corneal opacity, return of normal vision tests like menace gesture and ability to navigate the obstacle course. Success rate of different surgical techniques was evaluated on the basis of healing corneal ulcer. Incidence of intra-operative and post-operative complications was also recorded.

Statistical Analysis

Mean and Standard Error of various parameters were worked out using SPSS 20 software.

CHAPTER IV

RESULT AND DISCUSSION

Study was conducted on 28 eyes of 27 dogs with the history and clinical symptoms of ulcerative keratitis or corneal ulceration presented to the Department of Veterinary Surgery and Radiology, GADVASU, Ludhiana from the period of March 2016 to May 2017. All animals undergoing surgical treatment were selected randomly after complete ophthalmological, bacteriological and corneal impression smear examination. Recorded results have been presented and discussed below:

Age wise distribution of animals affected with corneal ulcers

Mean age of the animals in the study was 1.9±1.7 years (1month to 9 year). In the study highest incidence rate of corneal ulceration was recorded in age group of 1 to 2 years (40.74% ; 12/27), followed by age group of less than 1 year (33.33%; 9/27), age group of 2-3 year (18.5%; 5/27) and age group of 4 year and above (7.40%; 2/27). About 90% of animals under study were below 3 years of age (Table 2). Recorded observation was similar to the findings of Kim *et al* (2009) who also reported highest incidence of ulcerative keratitis in dogs with age group less than 3 year.

Table 2: Age wise distribution of animals affected with corneal ulceration

Age group	No. of cases	Percentage (%)
< 1 years	9	33.33%
1-2 years	11	40.74%
2-3 years	5	18.51%
> 4 years	2	7.40%

Table 3: Mean age wise distribution of affected animals in different treatment groups

Groups	Conjunctival pedicle graft	Conjunctival bridge graft	Amniotic membrane graft	Beaver blade debridement	Cotton swab debridement
Mean age (years)	2.17±1.39	2.89±3.19	0.77±1.03	1.58±0.49	1.70±0.57

Less sensitive cornea and playful nature of young dogs made them more prone to ocular trauma. There was no age related significant difference found in different treatment groups (Table 3). Age group of 1 to 3 year showed highest incidence of corneal ulceration (92.5%). Similar observation were given by Murphy *et al* (2001) and Ramani *et al* (2013) who stated that animals of 1 to 3 years were more prone to corneal ulceration. However, Moore (2003) reported high incidence of corneal ulceration in middle aged dogs with mean age of 8.2 years. Venugopal (2011) observed 79% incidence of corneal ulceration in age group of less than 1 year. In present study 33.33% incidence was reported in age group less than 1 year. In contrasts to above reports Wilkie and Whittaker (1997) were of view that dogs of any age could be affected with corneal ulceration.

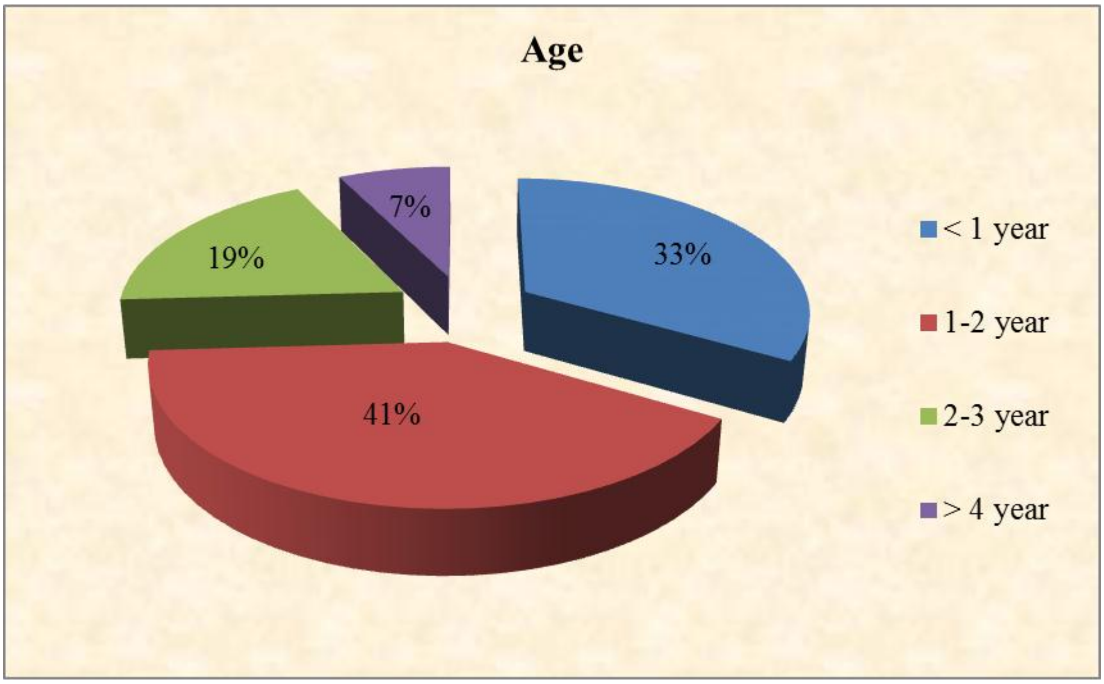


Fig. 5: Age wise distribution of animals affected with corneal ulcers

Sex wise distribution of animals affected with corneal ulcers

Corneal ulceration was reported in 18 male (66.66%) and 9 female (33.33%) dogs. Moore (2003) and Ramani *et al* (2013) also found high incidence of corneal ulceration in male dogs (67%) as compared to female dogs (33%). However, Wilkie and Whittaker (1997) and Murphy *et al* (2001) reported no sex related incidence of epithelial defects and stated that dogs of any sex were equally prone to corneal ulceration. In another study, Ramani *et al* (2012) reported 60.2% incidence of corneal ulceration in male dogs. Stanley *et al* (1998) found high incidence of corneal ulceration (57%) in male dogs than female dogs (43%). High predisposition of corneal ulceration in male dogs may be attributed to their vicious and fighting nature lead to frequent traumatic ocular injuries.

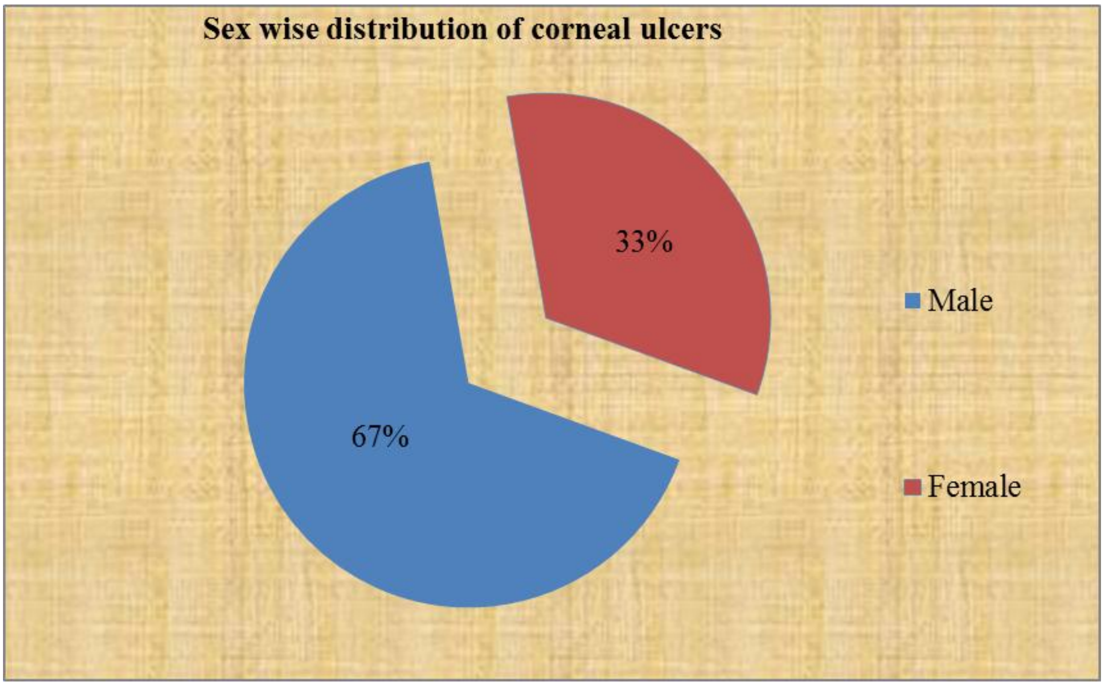


Fig. 6: Sex wise distribution of animals affected with corneal ulceration

Breed wise distribution of animals affected with corneal ulcers

Highest incidence of corneal ulceration was recorded in Pug breed (85.18%; 23/27), followed by Spitz, Dachshund, Toy Pomeranian, and Lasha Apso each (3.70%; 1/27) (Table 4). Findings were in accordance with the earlier study of Ramani *et al* (2012) and Ramani *et al* (2013) who reported highest incidence of corneal ulceration in Pug breed followed by Spitz, Non-descript, Boxers and Labrador retriever. Jose (2004) found that corneal ulceration was common ophthalmic condition of Spitz (51.85%), followed by other breeds like non-descript (22.22%), Lhasa apso (7.41%), Pug, Great Dane and German shephered. Thomson (2007) reported that brachycephalic breeds with pushed-in faces, such as Shih Tzu, Bulldog, French bulldog, Lhasa apso, Pekingese, and Pug were more prone to ulcerative keratitis. In the present study two brachycephalic breeds e.g. Pug (23) and Lhasa Apso (1) were reported to be affected with corneal ulceration. Relatively lagophthalmos eye of pug breed along with subnormal corneal sensitivity, less blinking frequency, central thinning of the pre-corneal tear film (Kern 1990) making it more susceptible to corneal trauma which was the most attributing cause of ulceration in present study.

Table 4: Breed wise distribution of animals affected with corneal ulcers

Breeds	Groups					No. of animals affected	Percentage (%)
	Conjunctival Pedicle graft	Conjunctival Bridge graft	Amniotic membrane graft	Debridement with beaver blade	Debridement with cotton swab		
Pug	5	5	3	6	4	23	85.18%
Daschund	1	0	0	0	0	01	3.70%
Toy Pom	0	0	0	0	1	01	3.70%
Lasha apso	0	1	0	0	0	01	3.70%
Spitz	0	0	1	0	0	01	3.70%
Total	**6**	**6**	**4**	**6**	**5**	**27**	**100%**

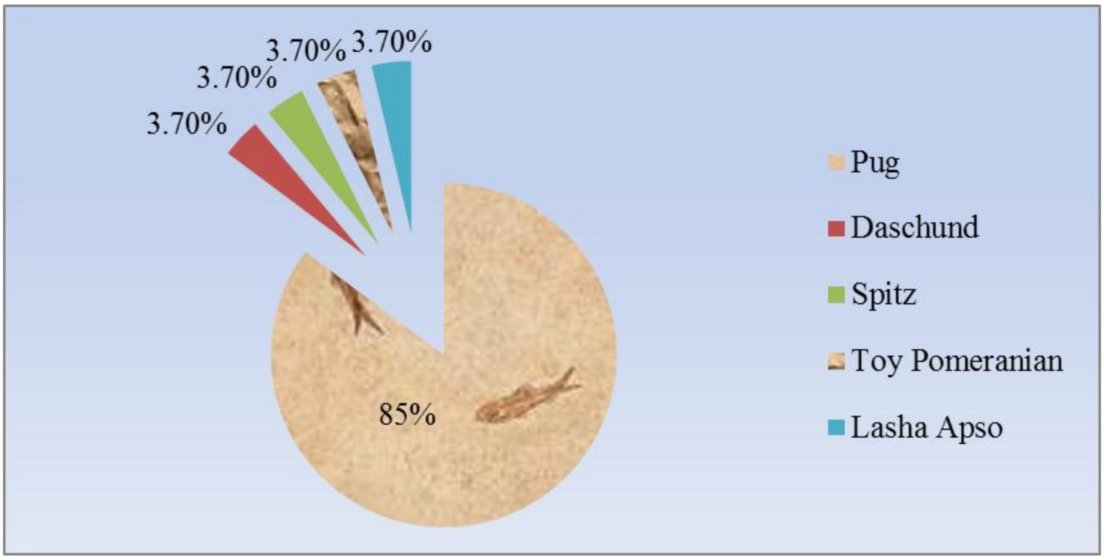


Fig. 7: Breed wise distribution of animals affected with corneal ulcers

Aetiology of corneal ulcers

There are numerous factors responsible for pathogenesis of corneal ulceration which setup failure of corneal defence mechanism and healing of epithelial defects (Stanley *et al* 1998). In present study traumatic injury to anterior epithelial (53.57%; 15/28), keratoconjunctivitis sicca (28.57%; 8/28), bacterial inflammation of cornea (14.28%; 4/28) and melachezzia infection (3.14%; 1/28) were the most attributing aetiological factors for corneal ulcers (Table no.5). These observations were supported by Moore and Nasisse (1999), Moore (2003) and Kim *et al* (2009) who found that lagophthalmos, bacterial infection, nasal fold trichiasis and trauma were the most important aetiologies for corneal ulceration. Other possible known causes for corneal ulceration were decreased tear production and facial fold trichiasis (Hvenegaard *et al* 2011). In present study KCS was reported as causative factor for ulceration. In eyes having reduced tear output, it was seen that ulcer were superficial and possess mild degree of corneal scarring and neovascularisation. One case of descemetocele eye was observed in 9 year old male Lasha apso. In this breed long hair projected down over eye induce progressive irritation over eyes. Frequent pawing over eyes to relieve irritation may lead to ocular trauma resulting in descemetocele eye. Bentley *et al* (2001) reported that abnormality in adhesion process between corneal layers may be responsible for pathogenesis of indolent ulcer. Fungal keratitis caused by Melachezzia was of rare occurrence. Melachezzia is an opportunistic organism having predilection on skin folds and middle ear of dog.

Table 5: Important aetiology factors reported in animals affected with corneal ulceration

Causative factor	No. of eyes affected	Percentage (%)
Traumatic injury	15	53.57
Decreased tear production/ KCS	8	28.57
Bacterial infection	4	14.28
Melachezzia infection	1	3.57

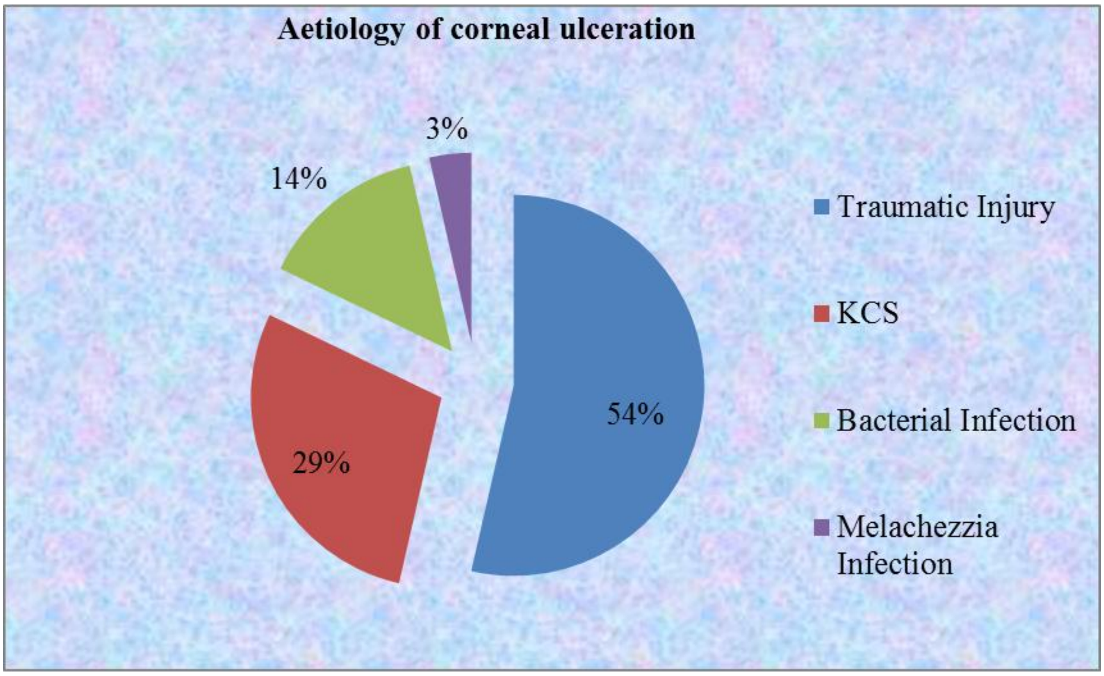


Fig. 8: Important aetiology factors reported in animals affected with corneal ulceration

Clinical signs associated with corneal ulceration or ulcerative keratitis

Mild to moderate degree of blepharospasm along with varying degree of conjunctival congestion was present in all cases (28/28; 100%). Mild to moderate degree of corneal opacity in terms of corneal oedema (28/28; 100%) and corneal neovascularization (pannus) (5/28; 17.85%) were also noticed. Other clinical sign like ephiphora, ocular discharge, creamy white deposit over descetmetocele, blood clots, irritation and pawing at affected side of eye were observed. These findings were in accordance with the observations of Whitley (2000), Croix *et al* (2001), Hvengaard *et al* (2011), and Singh *et al* (2016) who observed ocular hyperaemia, pain, photophobia, blepharospasm accompanied by purulent or mucopurulent ocular discharge. In another study Soontornvipart *et al* (2003) observed blepharospasm, protrusion of the nictitating membrane, epiphora and neovascularization of cornea in animals sufferings from corneal ulceration. Pain was not the evident clinical sign in present study and majority of animals did not exhibit this sign. This observation was supported by the study of Kern (1990) and Thomson (2007) who opined that brachycephalic breeds have less sensitive corneas and have high threashold of ocular pain. Brachycephalic breeds thereby, may display minimal signs of ocular pain. Melting ulcer, visible as central bulging and melting of anterior corneal epithelium was diagnosed in three eyes. Corneal sequestrum visualized as brown to black lesion of corneal necrosis at center with moderate corneal neovascularization was diagnosed in one pug dog. Bouhanna *et al* (2008) diagnosed corneal sequestrum in cats seen as central area of corneal necrosis with neovascularization. Neovascularization of cornea characterised as new blood vessel ingrowth from limbus to cornea denotes delayed and complicated corneal ulcer (Kern 1990).

Location based classification of corneal ulcers

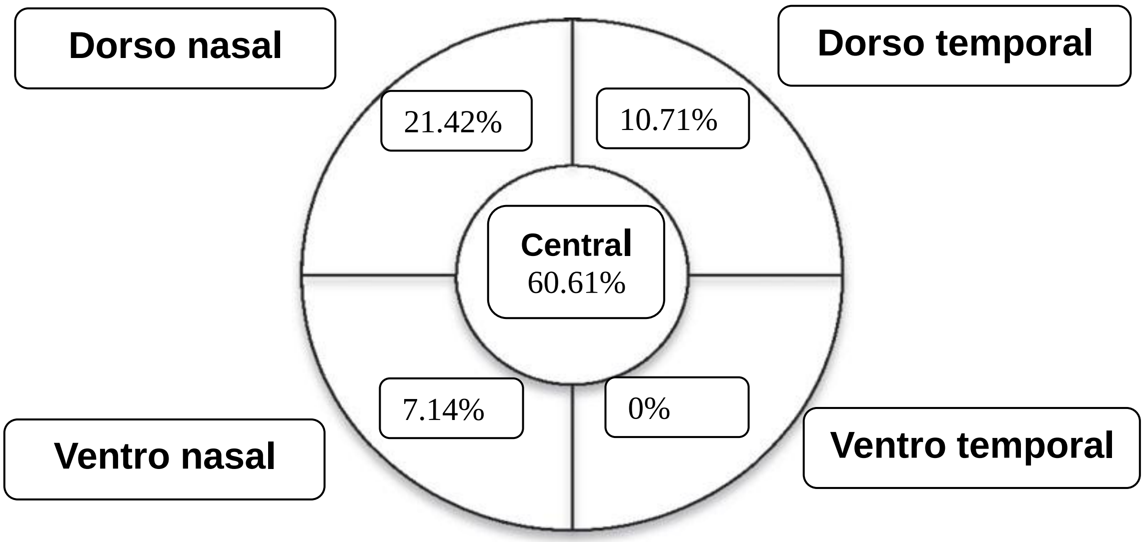
In present study, 28 eyes were broadly classified into four groups depending upon the location of ulcer on cornea (Table 6). Out of 28 eyes, 17 eyes had centrally located corneal ulcer, followed by dorsonasal ulcers (n=6), ventro nasal (n=2) and dorso temporal ulcer (n=3). These finding were supported by study of Dorbandt *et al* (2015) who also found high percentage of centrally located corneal ulcers in dogs. High percentage of centrally located corneal ulcers might be due to exophthalmia which prevent normal palpebral apposition and lead to lagophthalmos and uneven distribution of tear film at central region of cornea.

Table 6: Location based classification of corneal ulcers

Location of ulcer	No. of eyes affected	Percentage (%)
Central ulcer	17	60.61
Dorso-nasal ulcer	6	21.42
Dorso-temporal ulcer	3	10.71
Ventro nasal ulcer	2	7.14
Ventro-temporal ulcer	0	0

Table 7: Location based classification of corneal ulcers in different treatment groups

Surgical procedure used	Central	Dorso nasal	Dorso temporal	Ventro nasal
Conjunctival pedicle graft	6	1	0	0
Conjunctival bridge graft	2	2	1	0
Amniotic Membrane graft	4	0	0	0
Debridement with beaver blade	2	1	2	1
Debridement with sterile cotton swab	3	2	0	0



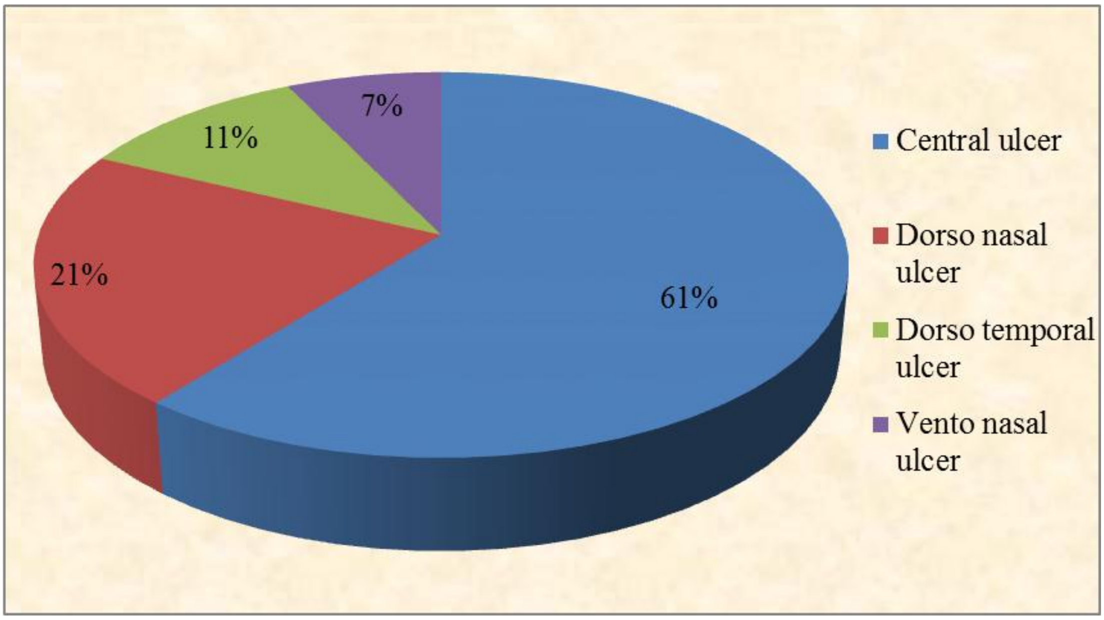


Fig. 9: Location based classification of corneal ulcers

Neuro Ophthalmic functioning reflexes

Neuro ophthalmic reflexes were evaluated on the basis of outcome of basic visual functioning tests like menace reflex, pupillary light reflex (PLR), palpebral reflex and cotton ball test (Table 8).

Table 8: Neuro Ophthalmic functioning reflexes (n=28)

Observations	Palpebral reflex	Menace Reflex	Pupillary light reflex	Cotton ball test
Absent (-)	-	9	15	15
Sluggish to absent (+)	-	5	2	3
Normal to sluggish (++)	-	4	1	-
Normal (+++)	28	10	10	10

Palpebral reflex: All 28 eyes were positive for palpebral reflex (100%). This test determines the integrity of maxillary branch of fifth cranial nerve (trigeminal nerve) and auriculpalpebral branch of seventh cranial nerve (facial nerve) through the pupil. However, the importance of palpebral reflex is questionable as Ofri (2008) was of view that palpebral reflex can be present in apparently blind animals

Menace reflex: Out of 28 eyes, 10 eyes (35.71%) showed normal menace reflex, 4 eyes (14.28%) showed normal to sluggish menace reflex and 5 eyes (17.85%) exhibited sluggish to absent menace reflex. In 9 eyes (32.14%) no response to menace reflex was seen. Menace test represents the functioning of optic nerve (CN-II, sensory) and facial nerve (CN-VII, motor). It is a subcortical reflex arising from the sudden stimulation to visual system (such as foreign body moving toward eye). Menace reflex lead to reflex closure of palpebral fissure and turning of head away from the noxious stimulus. Observations reported in present study were in accordance with the findings of Felchle and Urbanz (2001) and Bussieres *et al* (2004) who used menace reflex for confirmation of visual function and outcome.

Pupillary light reflex: PLR was found positive in 10 eyes (35.71%) and 15 eyes (53.57%) showed no response to pupillary light reflex. One eye (3.57%) has normal to sluggish PLR while 2 eyes (7.14%) exhibited sluggish to absent PLR. Pupil size of eye under direct bright light source was slightly smaller than the contralateral pupil size. This was considered as normal or satisfactory pupillary light reflex.

Extensive corneal opacity due to corneal oedema, deep and large sized epithelial defect prevents the light to pass through pupil and thereby prevent the pupil constriction. Therefore, majority of animals showed absent pupillary light reflex (Cullen and Grahn 2005). Findings in present study were in accordance with the observations of Felchle and Urbanz (2001) who used pupillary light reflex for confirmation of visual function and corneal transparency.

Cotton ball test: CBT was positive in 10 eyes (35.71%) and was absent in 15 eyes (53.57%). Three eyes (10.71%) showed sluggish to absent cotton ball test. Cotton ball test was considered positive when the dog followed the object to the floor (Maggs 2008). CBT determines the functioning of facial and abduces nerve. Martin (2001) was however, view that repeated trials of cotton ball test derived negative result as animals gets bored with cotton ball test. Therefore, this should be performed carefully to derive better results.

Fluorescein dye test

Fluorescein dye was used to detect corneal, conjunctival epithelial defects and pre corneal tear film deficiencies (Slatter 1990). Fluorescein sodium stain was taken up by exposed corneal stroma, therefore, defining the corneal ulcer margins as green (Singh *et al* 2016). In present study 22/28 eyes (78%) were positive for fluorescein dye test while 6/28 eyes (22%) do not take fluorescein dye staining due to its descetmetocele nature. Descemetocele is deep corneal lesion with protrusion of Descemet’s membrane and corneal endothelium followed by complete loss of epithelium and stroma (Featherstone and Stanley 2002). Intact healthy corneal epithelium and Descemet’s membrane did not took fluorescein stain (Felchle and Urbanz (2001). But whenever, corneal integrity is lost, the dye will enter water soluble corneal stroma and stains the intracellular spaces (Kern 1900; Slatter 1990). In case of indolent ulcers fluorescein dye migrating under the loose flaps of epithelium and thereby aids in the diagnosis of ulcer by staining the surrounding anterior stroma. Similar statement was given (Moore 2003; Janssens 2007) who stated that dye migrating under the loose flaps of epithelium, appears larger than the actual size. Singh *et al* (2016) also used fluoresceine sodium dye for detection of corneal ulceration and lesion was observed with naked eye or with the aid of magnification for staining (Strubbe and Gelatt 1999).

Schirmer's tear test

Schirmer tear test (STT) is one the most common test used for the evaluation of tear production. It is performed by placing SST strips in medioventral palpebral *cul-de-sac* of eye for one minute. In present study mean STT value for right and left eye was 14.70±4.51 mm/min and 15.67±3.99 mm/min respectively. STT should be performed before instillation of any topical medication and induction of general anaesthesia (Munro 2001 and Morreale 2003) because atropine topically or systemically, alone or in conjunction with general anesthesia significantly decreases tear production (Ludder and Heavner 1979, Hollingsworth *et al* 1992). Topical solutions may falsely increase STT values. Fear experienced by animal also increases sympathetic stimulation and falsely decreases STT values (Kaswan 1995). Gelatt (1991) suggested normal tear production for canine eye in the range of 15-25 mm/min. Kotani (2001), Thangamuthu and Varshney (2002) recorded normal range of tear production for canine eye 21.3±3.8 mm/min and 22.54±0.41mm/min respectively. Estimated STT value of the study (Right eye: 14.70±4.51 and left eye: 15.67±3.99 mm/min) were lower than normal tear production range therefore, it is suggestive of keratoconjunctivitis sicca (KCS) which could be the inciting cause of corneal ulceration. Kim *et al* (2009) also found that KCS was the inciting cause of corneal ulceration. It has been also reported that clinically normal animals may have STT values as low as 5 mm/min therefore, the interpretation of the values should be done in light of clinical signs (Bowersox and Criox 2001). There was no significance difference found in between different treatment groups. STT values in different treatment groups did not vary significantly.

Table 9: Mean STT value of eyes subjected to different treatment protocols

Surgical procedure used	**STT value (mm/min)**	
	Right eye	**Left eye**
Bulbar Conjunctival pedicle graft	15.00±4.43	15.17±3.31
Bulbar Conjunctival bridge graft	13.00±6.32	15.50±1.38
Human Amniotic Membrane graft	12.50±2.89	10.00±5.10
Surgical debridement with beaver blade no.# 64	16.67±5.16	19.17±2.99
Surgical debridement with sterile cotton swab	15.80±1.30	16.80±2.05
Mean STT value (mm/min)	14.70±4.51	15.67±3.99

SPECIAL DIAGNOSTIC TEST

Fig. 1: Schirmer tear strips

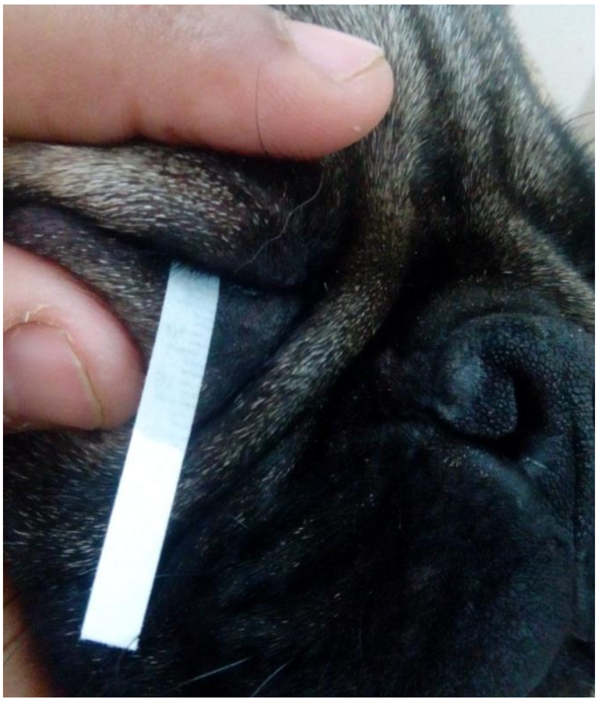


Fig. 2: Measurement of tear production using Schirmer's strip in a dog

Fig. 3: Fluorescein dye strips

Fig. 4: Dorso-temporal ulcer highlighted by fluorescent eye

Corneal Microflora

Corneal swab cultures have been considered more sensitive method for bacterial speciation, susceptibility testing and prompt diagnosis for successful management of bacterial corneal infections (Strubbe and Gelatt 1999). In present study 28 corneal swab samples were processed for bacterial isolation. Twenty one swab samples (21/28=75%) were positive for bacterial growth on brain heart infusion agar (BHI) or nutrient agar (NA) media whereas seven (7/28=25%) swab samples did not show any bacterial growth. Main isolated micro flora from twenty one swab samples included: *Staphylococcus spp. (71%)*, *Bacillus spp. (19%)* and *Streptococcus spp. (10%)* (Table 6). Swab samples that showed no growth could be due to use of topical antibiotic before case presentation. Findings of present study could be supported by earlier study of Ramani *et al* (2013) who isolated *Staphylococcus spp*. (54%), *Escherichia coli* (17%) and *Bacillus spp*. (8%) form corneal swab cultures of dogs and Anoop *et al* (2015) who isolated *Staphylococcus aureus* (40%), *Enterococci* spp (16%), *Staphylococcus intermedius* (10%), *Corynebacterium* spp. (12%), *Klebsiella* spp. (7%), *Bacillus* spp. (4%), *E. coli* (4%) and *Actinobactor* spp. (6%) from the corneal swab cultures of dogs suffering from pigmentary keratitis. Ollivier *et al* (2003) isolated Staphylococcus spp. (40%), Streptococcus spp. (25%), including β-Streptococcus spp. (16%) and α -Streptococcus spp. (9%), Escherichia coli (5%), Corynebacterium (4%) and Klebsiella (1%) from the conjunctival swab cultures of dogs.

Moore and Nasisse (1999) opined that *Staphylococcus* spp., *Streptococcus* spp. and *Enterococcus* spp. were the opportunistic bacteria that can make ocular infections more severe as they produce a number of enzymes and enterotoxins that enhance the progression of the infection and destruction of the cornea. Resident (commensal) non-invasive ocular flora play crucial role in the ocular defence mechanism by inhibiting growth of pathogens by nutritional competition, occupying space and by secretion of active substances that inhibit transient flora (Gerding and Kakoma 1990). Ledbetter and Scarlett (2008) and Kecova *et al* (2004) opined that resident ocular pathogens and previous corneal abnormalities predisposes cornea to anaerobic bacterial infection in long standing application of antibiotics and corticosteroids. In light of above study it was considered that pathogenic organisms involved in corneal infection were originally the part of resident ocular microflora.

Antibiotic sensitivity profiling

Twenty one samples of bacterial growth were subjected to antibiotic sensitivity testing (table no.10). Most of the isolates were sensitive to tobramycin, gatifloxacin, neomycin and gentamicin antibiotics.

Table 10: ASP for mirobes isolated from corneal swab samples

No. of sample	Isolated bacteria	Antibiotic sensitivity profile
15	***Staphylococcus spp.***	Gentamicin, Tobramycin, Neomycin, Gatifloxacin, Ofloxacin, Ciprofloxacin, Amoxycilin, Ampicillin
04	***Bacillus spp.***	Tobramycin, Neomycin, Ofloxacin
02	***Streptococcus spp.***	Neomycin, Tobramycin, Gentamicin, Cephalothin,

Diagnostic impression smear cytology

Corneal cytology is a diagnostic tool used for diagnosis and assessment of treatment regimen for ocular conditions that does not respond to empirical therapy (Slatter 1990). Impression smear from 25 eyes (25/28) were made for cytological examination. Cytological finding were divided into inflammatory type *(17; 68%)* (table 11) and non-inflammatory type *(8; 32%)* (table 12)

Table 11: Cytological finding for inflammatory type of impression smear

No. of eyes	Percentage	Cytological findings
10	58.82%	Sloughed squamous epithelial cells from different layers of anterior epithelium of cornea and plenty of inflammatory cells mainly of neutrophils along with fibrin and keratin debris
06	35.29%	Bacteria: clumps of cocci and rods with or without neutrophils
01	5.88%	Yeast: predominant no. of Melachezzia and keratin debris.

MICROBIOLOGICAL FINDINGS

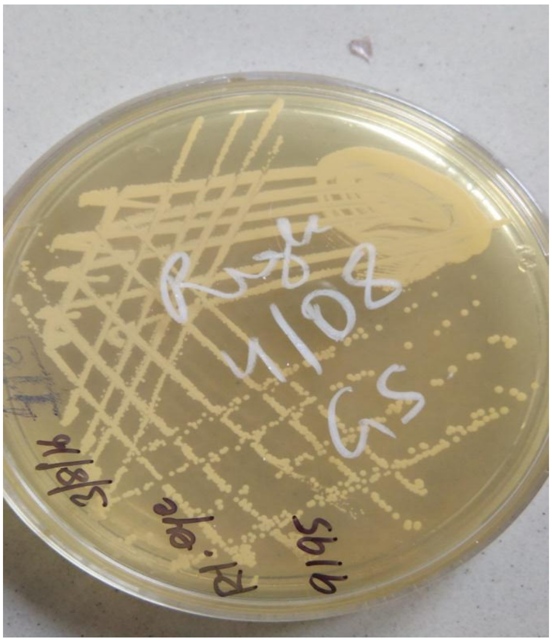


Fig. 10: Brain Heart Infusion agar plate showing colonies of Staphylococcus bacteria

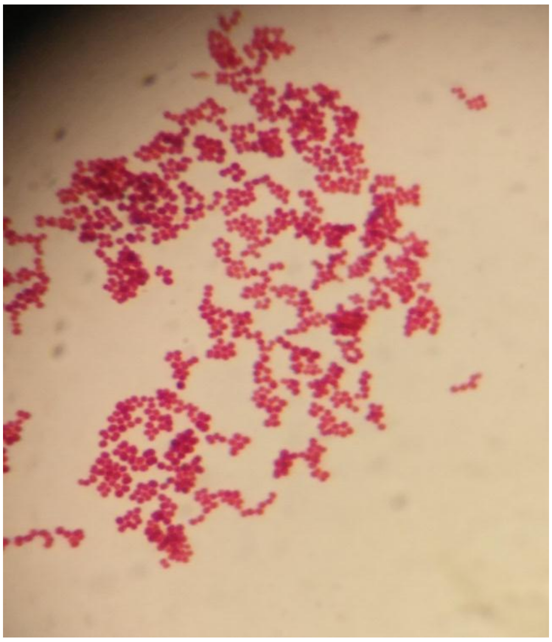


Fig. 11: Photograph showing- Bunch of grapes like appearance of bacteria suggestive of Staphylococcus spp. (gram staining X 100x)

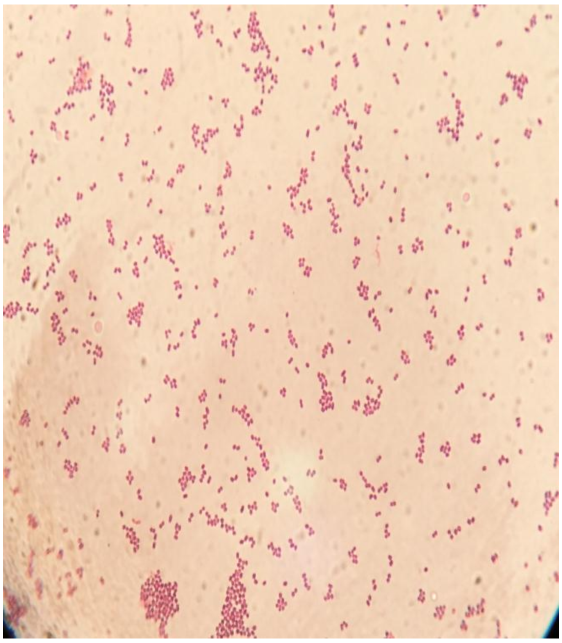


Fig. 12: Photograph showing-*Staphylococcus* spp. bacteria (gram staining)

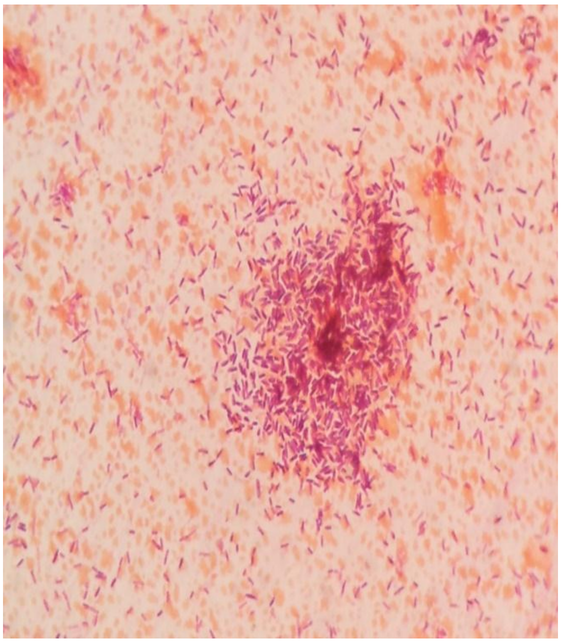


Fig. 13: Photograph showing- Rod appearance of bacteria suggestive of bacillus spp.(gram staining x 100x)

CYTOLOGICAL FINDINGS (PLATE 1)

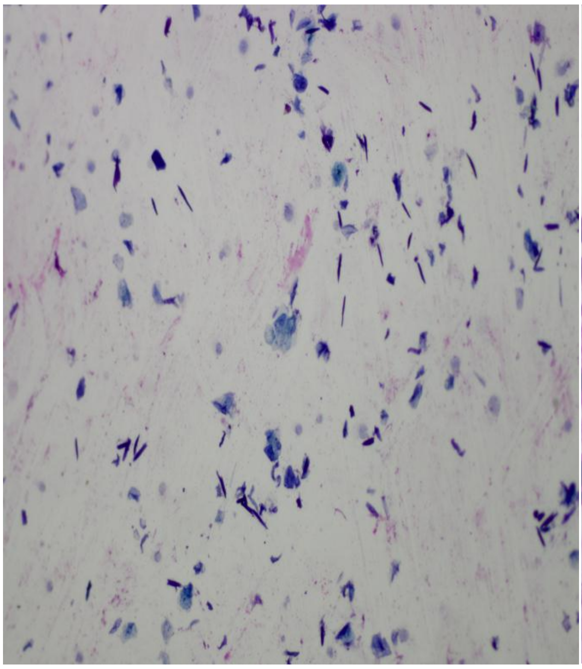


Fig. 14: Corneal smear showing-Superficial Squamous epithelial cells and neutrophils. Leishman stain x40X

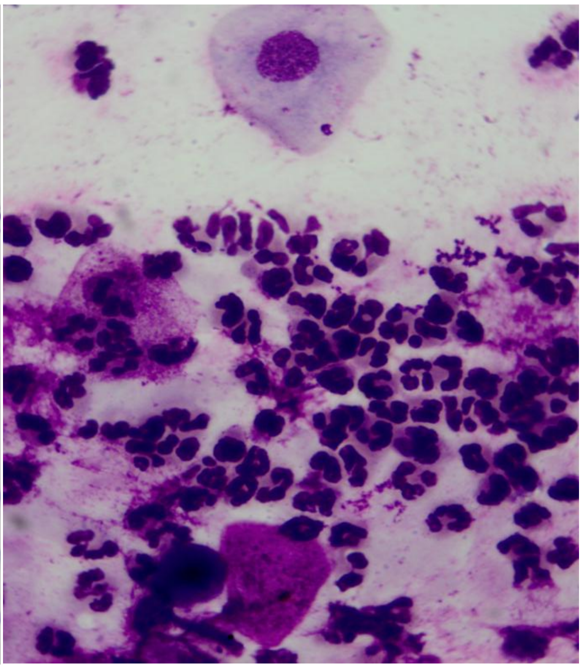


Fig. 15: Corneal smear showing-Superficial Squamous epithelial cells and predominance of neutrophils. Leishman stain x100X

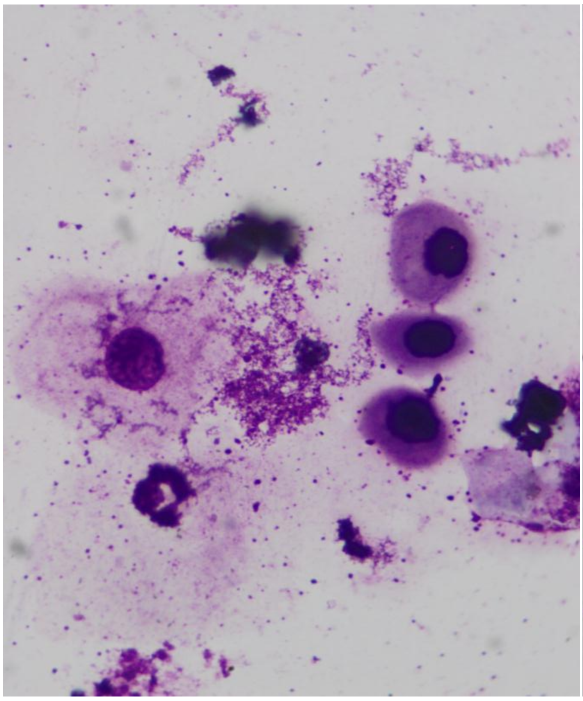


Fig. 16: Corneal smear showing-Superficial Squamous epithelial cells and neutrophils along with keratin debris Leishman stain x100X

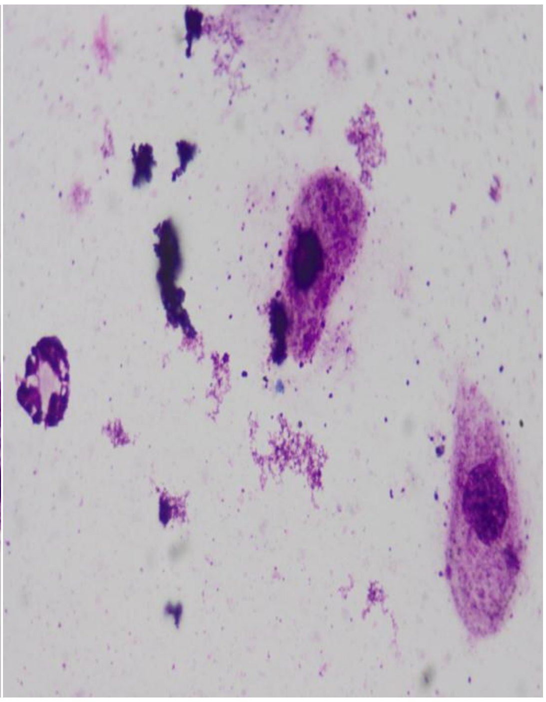


Fig. 17: Corneal smear showing-Superficial Squamous epithelial cells and neutrophils and keratin debris . Leishman stain x100X

CYTOLOGICAL FINDINGS (PLATE 2)

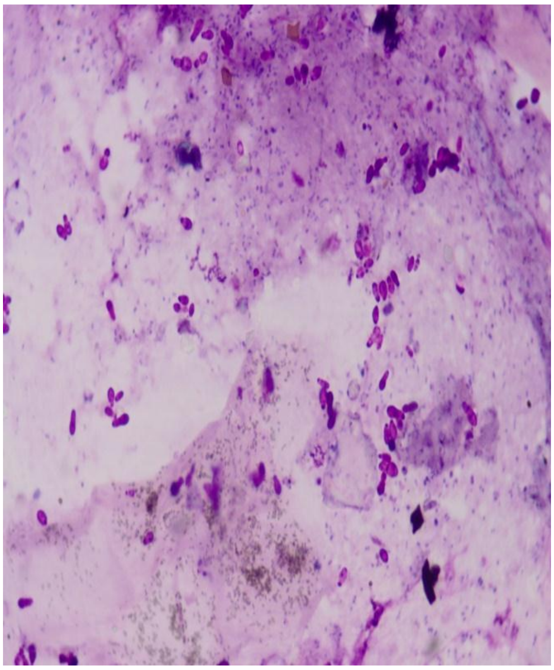


Fig. 18: Corneal smear showing-abundant number of melachessia organism and few neutrophils and clumps of bacteria. Leishman stain x100X

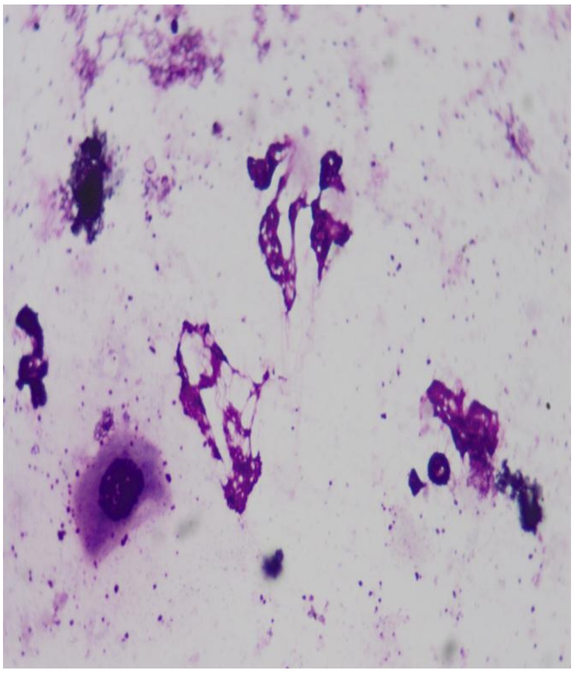


Fig. 19: Corneal smear showing-Superficial Squamous epithelial cells and degenerated neutrophils. Leishman stain x100X

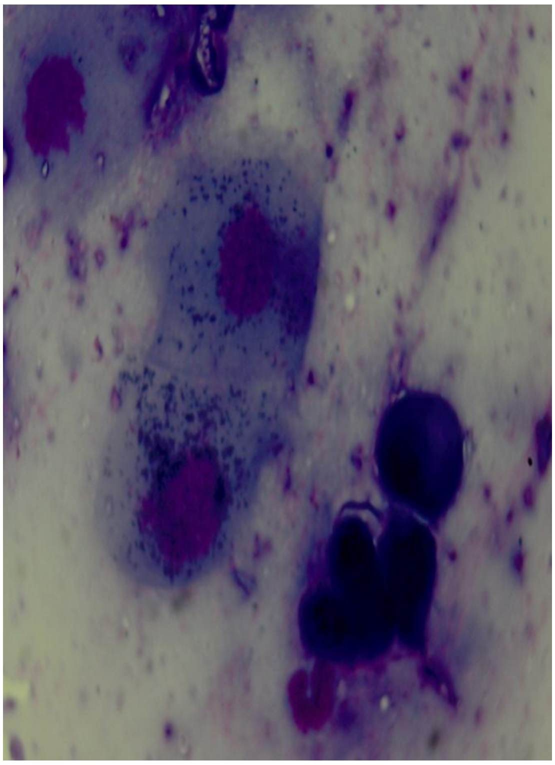


Fig. 20: Corneal smear showing- Squamous epithelial cells of deep layer and neutrophils and keratin debris. Leishman stain x100X

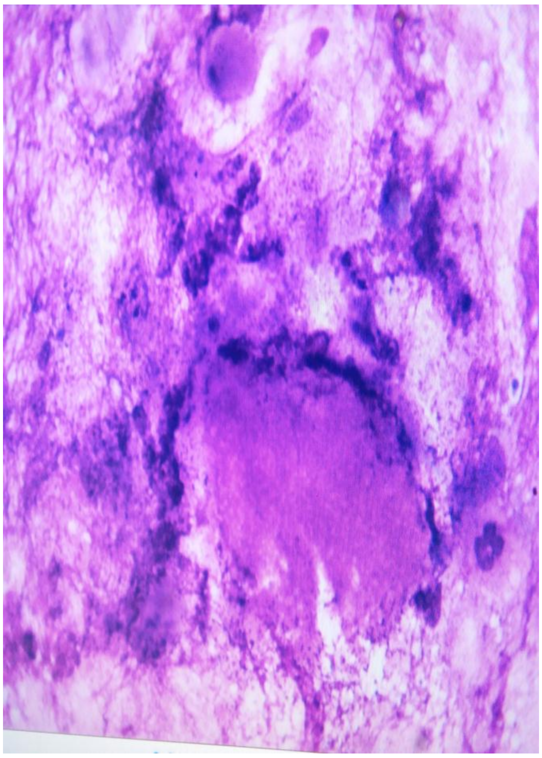


Fig. 21: Corneal smear showing- Superficial Squamous epithelial cells and karyorrhexis. Leishman stain x100X

Table 12: Cytological finding for non- inflammatory type of impression smear

No. of eyes	Percentage (%)	Cytological findings
08	32%	Admixture of fibrin threads and keratin debris with squamous epithelial cells from different layers of anterior epithelium of cornea without any inflammatory cells

Findings of the present study were in accordance with Anoop *et al* (2016) who studied impression smear cytology of canine cornea and observed the infiltration of neutrophils, degenerative changes, presence of necrotic debris and squamous epithelial cells as major cells in impression samples. Gerding *et al* (1988) found that non cornified superficial corneal epithelial cells, intermediate corneal epithelial cells, lymphocytes and polymorphonuclear neutrophils normal corneal cell as. In bacterial infection polymorphonuclear neutrophils, keratin debris, mucus, and bacterial clumps were predominant cells. Impression cytology is simple and reliable method of sampling corneal and conjunctival epithelium cells, which allows monitoring of the ocular disease process and grading of condition (Balicki *et al* 2011). It is a quick and non- invasive method of detecting the corneal pathology based on which treatment can be followed.

Haematological parameters

Various haematological parameters estimated were within the normal rerange (Table 13). These findings were in accordance with study of Chinchu (2010), Antonia (2014) and Anoop *et al* (2016).

Table 13: Mean±SE values of haematological parameters in animals suffer from corneal ulceration

Parameter		***Mean±SE***
Haemoglobin	(g/dl)	11.72±1.75
TLC	(10^3× /µl)	19.67±7.82
Platelet count	(10^3× /µl)	327.22±113.47
PCV	(%)	38.90±5.02
DLC	Neutrophils	79.62±11.01
	Lymphocyte	19.26±11.00
	Monocyte	00.22±00.64
	Eosinophil	01.40±02.06

Haemoglobin (Hb): The overall mean±SE Hb value of the study (11.72±1.75 g/dl) was within normal reference rage. Hb value for all treatment groups viz. conjunctival pedicle graft (13.00±0.43), conjunctival bridge graft (10.87±0.95), amniotic membrane graft (11.35±0.64), surgical debridement with beaver blade (11.55±0.62) and surgical debridement with cotton swab (11.74±0.91) were within normal range (11.9-18.9 g/dl) given by Aiello *et al* (2016).

Total leukocyte count (TLC): Mean±SE value of TLC for the study was ($19.67 \pm 7.82 \times 10^3$) which was slightly higher than normal reference range ($5\text{-}14.1 \times 10^3 \times$ /µl) (Aiello *et al* 2016). TLC count for different treatment groups viz. conjunctival pedicle graft ($14.83 \pm 6.25 \times 10^{3)}$, amniotic membrane graft ($16.68 \pm 3.26 \times 10^3$) were within normal range (Aiello *et al* 2016). TLC count for conjunctival bridge graft ($24.62 \pm 11.13 \times 10^3$), debridement with beaver blade ($22.24 \pm 5.73 \times 10^3$) and surgical debridement with cotton swab ($19.37 \pm 6.98 \times 10^3$) were elevated.

Platelets count: Mean±SE platelets count of the study was ($327.22 \pm 113.47 \times 10^3$) is within normal reference range i.e. ($211\text{-}621 \times 10^3 \times$/µl) given by (Aiello *et al* 2016). Platelet count for different treatment groups viz. conjunctival pedicle graft ($236.33 \pm 109.40 \times 10^3$), conjunctival bridge graft ($320.17 \pm 119.93 \times 10^3$), amniotic membrane graft ($357.25 \pm 29.06 \times 10^3$), surgical debridement with beaver blade no.#64 ($371.83 \pm 73.94 \times 10^3$) and surgical debridement with cotton swab was ($367.20 \pm 157.89 \times 10^3$) were within normal range.

Packed cell volume: Mean±SE PCV value of the study was (38.90±5.02) within normal reference range i.e. (35-57%) given by (Aiello *et al* 2016). Group wise estimated PCV value of conjunctival pedicle graft (38.00±4.71), conjunctival bridge graft (38.88±5.89), amniotic membrane graft (38.63±3.45), surgical debridement with beaver blade (38.77±5.62) and surgical debridement with cotton swab (35.90±6.05) were within normal range

Neutrophils: Mean±SE neutrophil count of the study was (79.62±11.01) within normal reference range i.e. (58-85%) given by (Aiello *et al* 2016). Group wise estimated neutrophil count value viz. conjunctival pedicle graft (79.50±8.75), conjunctival bridge graft (82.66±10.32), amniotic membrane graft (77.25±3.20), surgical debridement with beaver blade no. #64 (79.66±9.50) and surgical debridement with cotton swab (78.00±20) were within normal range.

Lymphocytes: Mean±SE lymphocyte value of the study was (19.26±11.00) within normal reference range i.e. (8-21%) given by (Aiello *et al* 2016). Lymphocyte count value for different treatment groups viz. conjunctival pedicle graft (21.33±8.36), conjunctival bridge graft (14.33±7.94), amniotic membrane graft (21.75±2.36), surgical debridement with beaver blade no.#64 (21.17±9.17) and surgical debridement with cotton swab was (18.40±21.33) were within normal range.

Surgical management of corneal ulcers

Corneal ulcer is one of the most common ocular ailment in companion animals. It can lead to blindness or loss of eye (Whitley and Gilger 1999, Ollivier 2003). It is considered to be a big therapeutic challenge in veterinary ophthamology. Stanley *et al* (1998) suggested different treatment protocols with variable success rate. Successful management and assessment of surgical repair of corneal injury depends upon the clinical signs, length of the laceration, location i.e. peripheral cornea vs. central cornea and depth of insult i.e. full thickness or partial thickness (Hollingsworth 2003). While treating corneal ulcers, first attempt should be made to eliminate causative factor, creating ideal environment for healing, prevention of progression of ulcer and providing ex situ or mechanical support to weaken cornea through surgical interventions (Rebhun 1983, Boruchoff and Foulks 1990, Dawson and Sanchez 2015). Surgical treatment of corneal ulcers are generally aimed with objectives like preservation of corneal integrity, minimizing lesions incompatible with functional vision and replacement of lost corneal tissue (Gelatt 1991). Various treatment options for corneal ulcers include conjunctival grafts (Boisjoly *et al* 1989, Dorbandt *et al* 2015), third eyelid flap (Helper 1981), amniotic membrane grafting (Gelatt 1991), corneo-scleral grafting (Slatter 1990), corneal transplantation (Severin 1995), epithelial debridement (Morgan and Abrams 1994). In present study five treatment protocols viz. Pedicle conjunctival graft (group I A), Bridge conjunctival graft (group I B), Amniotic membrane graft (group II) and debridement with beaver blade no.# 64 (group III A) and with sterile cotton swab (group III B) were used for surgical management of corneal ulcers.

Group I- Bulbar Conjunctival grafting

Group I-A--Bulbar pedicle conjunctival grafting

Bulbar pedicle conjunctival grafting was performed in seven eyes of six animals, followed by partial temporary tarsorrhphy. Tarsorrhphy suture were removed after 10 days and eyes were evaluated for surgical outcome. Clinical examination showed six eyes (n=6/7; 85 %) having intact graft to ulcer bed. In one eye (n=1/7; 15%) self-mutilated graft dehiscence on next day of procedure was observed. Marked neovascularization of conjunctival graft was noticed in all eyes. No blood vessel was seen invading the normal cornea. Wilkie and Whittaker (1997) stated that graft neovascularization was beneficial to recipient bed as it provide increased blood supply to the healing cornea that was not provided by other procedures like lamellar corneoscleral transposition or full thickness corneal graft. In study of Morgan and Abrams (1994) it has been reported that conunctival grafts provide immediate vascular supply to compromised cornea thereby, bringing blood associated immune components and growth factors, anticollagenase activity mainly (alpha 2 macroglobulin) and systemic antibiotics to the ulcer bed. Corneal oedema was evident in all eyes and observed around the periphery of needle bite. It was due to fact of needle penetration through the corneal endothelium. Healing of cornea was satisfactory along with mild to moderate corneal oedema. All eyes were evaluated on 30th post-operative day for stage of healing, extent of corneal oedema and level of neovascularization. It was observed that all ulcers were healed completely with marked subsided corneal oedema and satisfactory conjunctival graft neovascularization. High success rate (85.71%) was achieved in animals operated with bulbar conjunctival pedicle graft. Outcome was in accordance with the study of Wilkie and Whittaker (1997) and Soontornvipart *et al* (2003) who reported success rate of 90% and 93.18% respectively following the application of conjunctival pedicle grafts in dogs and cats.

Graft trimming was done after 45th post-operative days. Graft was trimmed from the base and left on ulcer bed. Six eyes regained vision with acceptable scar at ulcer bed up to last evaluation. Post-operative complications were noted as graft dehiscence (1), corneal oedema (6), mild keratitis (1), scar at ulcer bed (6) and corneal pigmentation (2).

Hollingsworth (2003) found that conjunctival pedicle grafting a much superior technique than other corneal surgery procedures. Conjunctival pedicle grafts helped in healing of corneal ulcers by preservation of corneal integrity, minimizing lesions incompatible with functional vision and replacement of lost corneal tissue (Holmberg

GROUP I-A: CONJUNCTIVAL PEDICLE GRAFTING (ANIMAL 1)

Fig. 25 Day 0 Pre-operative showing central corneal ulcer

Fig. 26: Day 10 Post-operative conjunctival grafting neovascularization

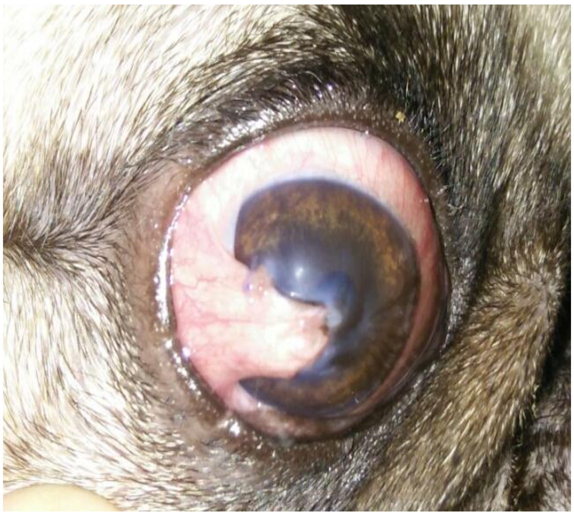


Fig. 27: Day 30 Photograph showing healed ulcer with minimum corneal oedema and scarring

Fig. 28: Day 45 Photograph showing trimmed graft left only at ulcer bed

Fig. 29: Day 60 Photograph showing corneal scar at ulcer bed with transparent cornea

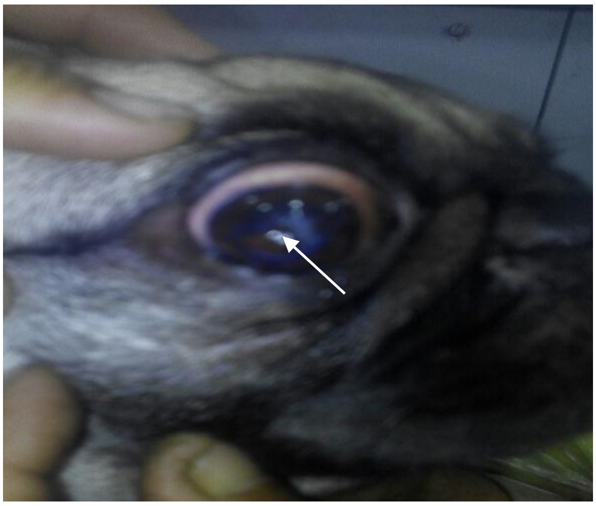


Fig. 30: Day 90 Photograph arrow depicting regressed corneal scar with satisfactory corneal transparency

GROUP I-A: CONJUNCTIVAL PEDICLE GRAFTING (ANIMAL 2)

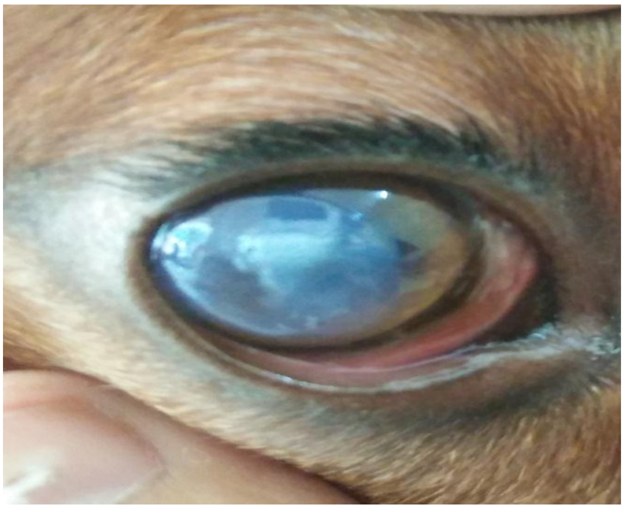


Fig. 31: Day 0 Photograph showing central melting of cornea

Fig. 32: Day 0 Photograph showing eye after debridement of central melting ulcer with cotton swab

Fig. 33: Day 5 Photograph showing ulcer refractory to heal after debridement and topical medication

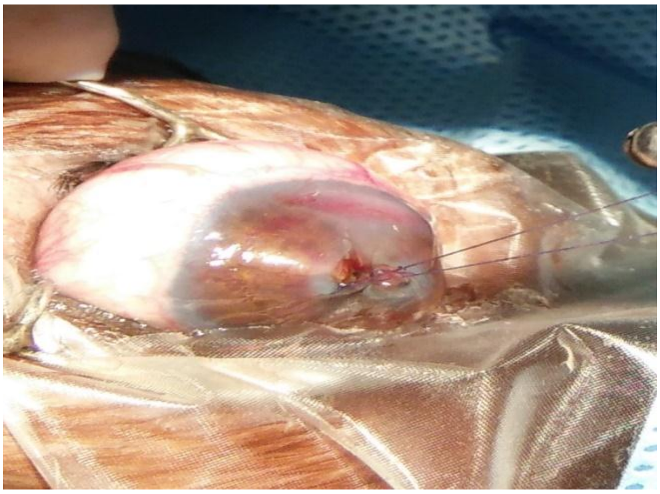


Fig. 34: Day 5 Photograph showing corneal suturing of same eye was

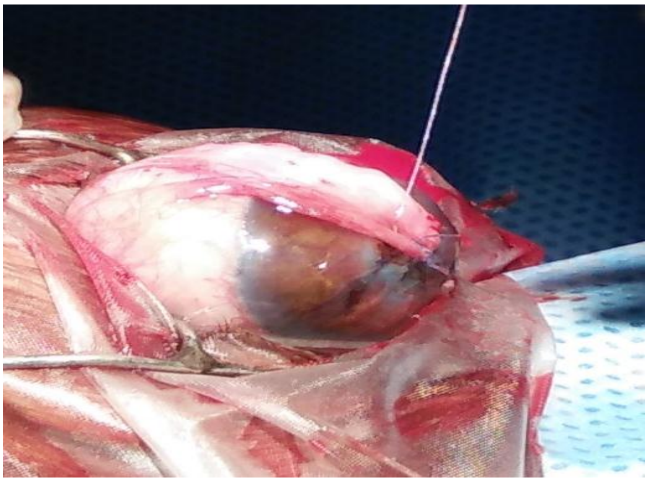


Fig. 35: Day 0 Photograph showing suturing of pedicle with cornea in same eye

Fig. 36: Day 10: Photograph showing evident graft neovascularization along with marked corneal oedema

Fig. 37: Day 30 Photograph showing marked subsided corneal oedema and graft neovascularization

Fig. 38: Day 45: Photograph showing regain of normal corneal transparency with regression of graft neovascularization

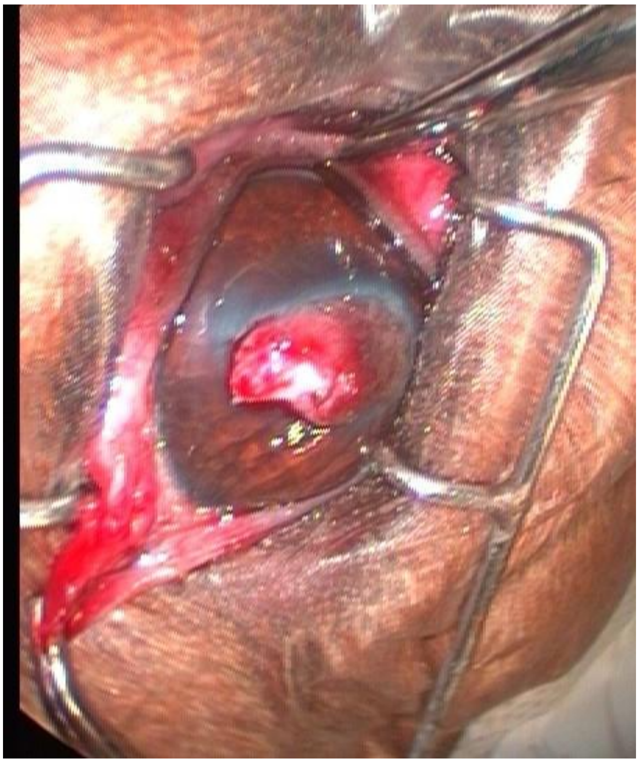


Fig. 39: Day 45 Photograph showing trimmed graft from base and left on ulcer bed

Fig. 40: Day 60: Photograph showing corneal scar at ulcer bed with oedema

GROUP I-A: CONJUNCTIVAL PEDICLE GRAFTING (ANIMAL 3)

Fig. 41: Day 0 Photograph showing cental corneal ulcer and procurement of pedicle

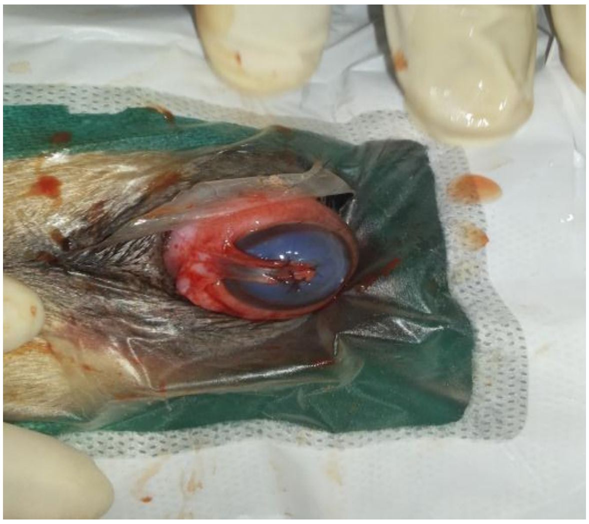


Fig. 42: Day10 Photograph showing suturing of pedicle with recipient bed

Fig. 43: Day 10 Photograph showing marked corneal oedema and graft neovascularization

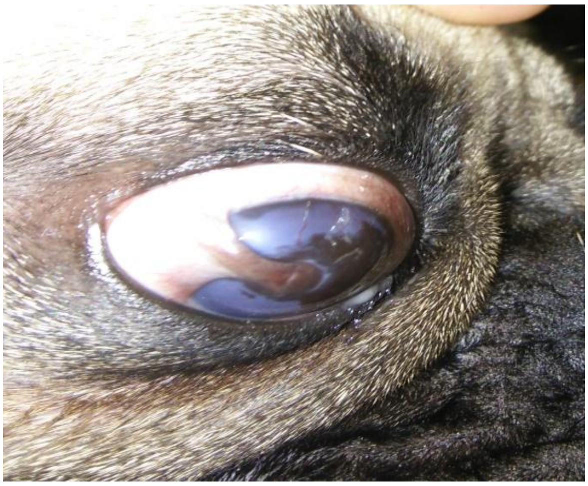


Fig. 44: Day 30 Photograph showing subsided corneal oedema and graft neovascularization

Fig. 45: Day 45 Photograph after trimming of graft from base and corneal scarring

Fig. 46: Day 90 Photograph showing regressed scar and satisfactory transparency

1981). Conjunctival pedicle grafts provide increased blood supply to the healing cornea that was not provided by other procedures like lamellar corneoscleral transposition or full thickness corneal graft (Wilkie and Whittaker 1997).

Since apart from covering ulcer bed, pedicle graft cover only small area of normal cornea thus allowed maximum visualization and evaluation of cornea. It also allowed visualization of anterior chamber of treated eye to ophthalmologist. It thereby, helped in monitoring of progression of ulcer so that the complication can be dealt at an early possible time. Similar observation was given Gilger *et al* (2007) who opined that pedicle grafting technique gives be better visualization of cornea.

Table 14: Complications recorded in animals of (Group I-A) treated by conjunctival pedicle grafting

Complication	Graft dehiscence	Corneal oedema	Corneal scaring	Pigmentation
Observation	Graft mutilation was seen in one animal due to self-trauma on 2nd day of procedure.	Corneal oedema noticed up to 10th post-operative day. Subsided up to 30th post-operative day.	Observed in all cases after graft excision. Lighting of scarring with time was seen.	Pigmentation was observed up to last evaluation.
No. of eyes affected	1	6	6	2

Group IB Bridge or bipedicle conjunctival graft from bulbar conjunctiva

Bulbar bridge conjunctival grafting was performed in six eyes of six animals. Grafting was accompanied by partial temporary tarsorrhphy. Tarsorrhphy sutures were removed after 10 days and eyes were evaluated for outcome. Three eyes (50%) had intact graft to ulcer bed. In three eyes (50%) graft mutilation was observed on 4th, 7th and 14th post-operative day respectively. Conjunctival graft neovascularization was evident on 10th post-operative day. Due to graft dehiscence in one eye bridge conjunctival graft was changed to conjunctival pedicle graft on 4th post-operative day. Animals which developed graft dehiscence were again evaluated on 15th post-operative day. It was observed that small piece of graft was still covering ulcer bed and ulcer was at good healing stage along with marked neovascularization. In one eye graft dehiscence was observed on 14th post-operative day. It was observed that graft was still covering the ulcer bed however, graft was torn from the base on both sides. On 30th post-operative day completely healed corneal ulcer, marked subsided corneal oedema and conjunctival neovascularization were observed.

Four eyes (66.66%) resumed to vision with acceptable scar at ulcer bed up to last evaluation. In one case, bridge or bipedicle grafting yielded the thick granulating tissue, covering almost 70% of cornea that hampered the vision. Bussieres *et al* (2004) observed similar complication but vision was restored in their study. Success rate in terms of restoration of functional vision was achieved in 66.66% animals. Kim *et al* (2009) reported success rate of 100% in superficial corneal ulcers and 55% in deep corneal ulcers with conjunctival flap grafting. It was observed that two eyes that failed to restore vision were because of the descemetocele nature of the lesion. However, cosmetic appearance of both eyes was preserved.

Hollingsworth (2003) suggested different conjunctival flaps for corneal surgery including conjunctival pedicle graft, conjunctival bridge graft, transconjunctival island graft, advancement graft and total conjunctival graft. These flaps provided direct blood supply to the corneal wound (Dice 1981) and raised corneal temperature, thereby facilitating corneal healing by increasing cellular metabolism of corneal cells (Helper 1981). Kim *et al* (2009) reported that conjunctival flap helped to heal deep corneal ulcers within 28 to 40 days. Bridge grafting was indicated in large diameter corneal defects where conjunctival pedicle graft was unable to cover large defects completely (Gellat 1991). Bussieres *et al* (2004) used bridge conjunctival flap in conjunction with small intestine sub mucosa (SIS) graft for stromal abscesses in horse. Bridge graft in this patient yielded very thick granulation tissue however, vision was restored in animal. In another study conjunctival grafting technique was found much easier to perform and was less time consuming than lamellar keratoplasty (Laus *et al* 1999). Conjunctival flaps were indicated for repair of large defects like recurrent corneal erosions and deep or progressive ulcers (Maggs 2008). Ultimate goal of this graft was to cover the defect and its surroundings, achieving conjunctival to epithelium apposition and providing mechanical support to weak cornea (Wilkie and Whittaker 1997).

GROUP I-B: CONJUNCTIVAL BRIDGE GRAFT (ANIMAL 1)

Fig. 47: Day 0 Photograph showing centraly located descetmetocle

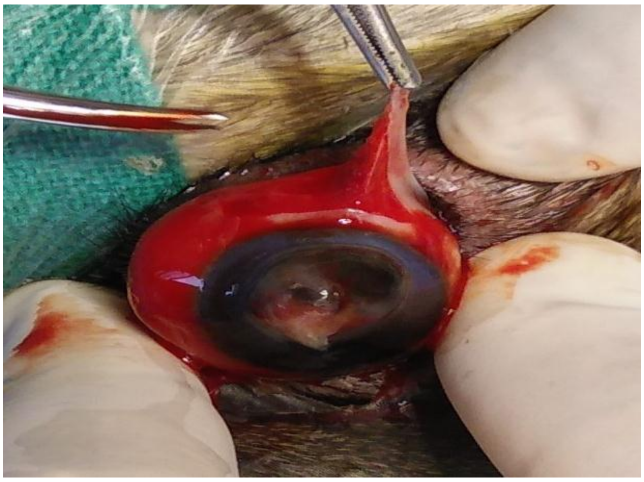


Fig. 48: Photograph showing procurement of pedicle from superior conjunctiva

Fig. 49: Photograph showing two sutured pedicle with cornea

Fig. 50: Day 10 Photograph showing evident graft neovascularization and corneal oedema

Fig. 51: Day 30: Photograph showing healed corneal ulcer with regression in graft neovascularization

Fig. 52: Day 45: Photograph showing trimmed graft from base and graft is adhered to ulcer bed

GROUP I-B: CONJUNCTIVAL BRIDGE GRAFT (ANIMAL 2)

Fig. 53: Day 0 Photograph showing dorso-nasal corneal ulcer

Fig. 54: Day 0 Photograph showing exposure of bulbar conjunctiva and two parallel incision

Fig. 55: Day 0 Photograph showing translocation of bridge of conjunctiva over ulcer

Fig. 56: Day 4 Photograph showing mutilation of bridge graft due to self-trauma

Fig. 57: Day 15 Photograph showing due to graft dehiscence, bridge graft is converted into pedicle graft

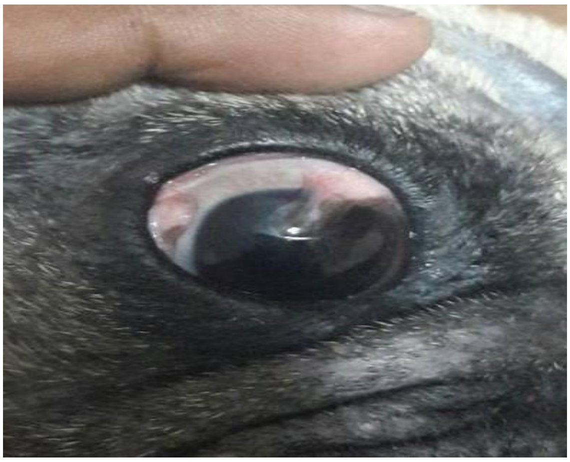


Fig. 58: Day 30 Photograph showing adherence of graft to ulcer bed

Graft dehiscence (3), corneal oedema (6), mild keratitis (1), scar at ulcer bed (4) and corneal pigmentation (2) were the most significant post-operative complications in animals operated by bridge conjunctival grafting technique. Corneal oedema was more evident on 10th post-operative day. However, it subsided markedly up to 30th post-operative day. It may due to fact of needle penetration through corneal endothelium might have lead to breach in endothelium defence mechanism. Therefore, corneal endothelium was no longer able to remove fluid from cornea results in corneal oedema as also opined by Gellat (2007). Maggs (2008) found that corneal opacity resulting from uncomplicated stromal wounds can be limited by use of corticosteroids, provided that infection has been controlled and an epithelial integrity was restored. However, in present study no corticosteroids were used because control of infection is always a challenging job in animals suffering from corneal ulcers.

Table 15: Complications recorded in animals of (Group I-B) treated by conjunctival bridge grafting

Complication	**Graft dehiscence**	**Corneal oedema**	**Corneal scaring**	**Pigmentation**
Observations	Observed in two eyes on 4th and 6th post-operative day due to self-mutilation.	Marked corneal oedema was seen on 10th post-operative day Oedema subsided markedly on 30th post-operative day.	Corneal scarring was seen in all animals. In one animal graft became excessive granulating tissue and hampered the vision.	Observed up to last evaluation.
No. of eye affected	2	4	4	2

Group II- Amniotic membrane grafting as overlay patch

Various types of amniotic membrane transplantation have been suggested e.g. glycerol-preserved equine amniotic membrane (Barros *et al* 1998); multilayer cryopreserved human amniotic membrane (Kruse *et al* (1999); equine amniotic membrane (Lassaline *et al* 2005); canine amniotic membrane (Kalpravidh *et al* 2009);

bovine freeze-dried amniotic membrane (Kim *et al* 2009); human amniotic epithelial cell culture (Wichayacoop *et al* 2009); frozen fresh amniotic membrane (Agraval *et al* 2017).

In present study dry human amniotic membrane (AM) overlay graft was applied in four eyes. Two layered human amniotic membrane graft was placed over ulcer without suturing or any tissue adhesive. It was followed by partial temporary tarsorrhphy. Size of the AM was kept in such dimension so that it covered the entire cornea. In one animal lateral canthotomy was performed to ease the grafting. After grafting canthotomy incision was sutured. Tarsorrhphy sutures were removed after 10 days and eyes were evaluated for outcome.

All animals treated by overlay amniotic membrane graft showed healed corneal ulcer within 11.25±2.50 days with complete epithelisation of cornea. This suggests that human amniotic membrane contains a number of growth factors that favour epithelial healing within short time period (Koizumi *et al* 2000). Moreover, basement membrane of human amniotic membrane facilitates the migration of epithelial cells that helps in re-establishment of adhesion process between newly generated epithelial cells and underlying basement membrane (Khodadoust *et al* 1968).

Human amniotic membrane transplantation was commonly used as multilayer membrane transplantation (Prabhasawat *et al* 2001; Rodrigues-Ares *et al* 2004). Number of layers of amniotic membrane to be used depends upon the dimension of ulcer (Solomon *et al* 2002). Rodriguez-Ares *et al* (2004) stated that increased number of layers of amniotic membrane significantly increase the epithelialization time of perforations. In the contrast of above study two layered amniotic membrane transplantation was performed in the present study. Amniotic membrane was applied over cornea in such a way, that the stromal side of amniotic membrane was faced the epithelial lining of cornea. In the earlier studies it was found that stromal side of AM helped to facilitate the epithelial cell migration, reinforces adhesion of the basal epithelium, promotes cellular differentiation, prevents apoptosis and thereby, significantly reducing the epithelisation time (Tourino *et al* 2004). Time to achieve complete epithelisation was 10 days in three animals and in one animal it was about 15 days. Mean corneal epithelisation time was observed 11.25±2.50 days

GROUP II: AMNIOTIC MEMBRANE GRAFTING (ANIMAL 1)

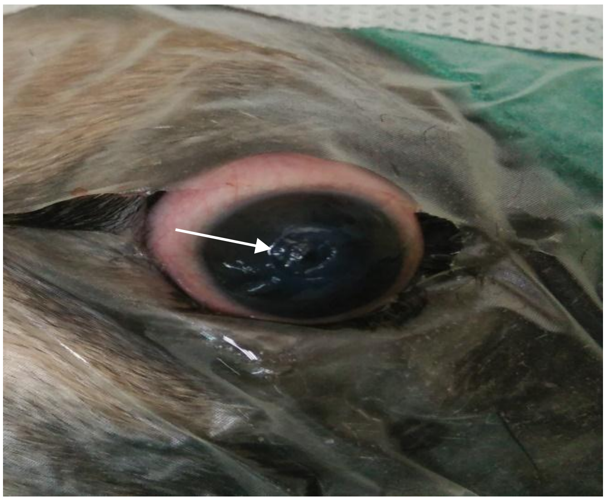


Fig. 59: Day 0 Photograph showing centrally located corneal ulcer

Fig. 60: Day 0 Intra-operative photograph showing application of amniotic membrane graft

Fig. 61: Day 0 Intra-operative photograph showing adhesion of amniotic membrane with cornea and careful closure of eyelids

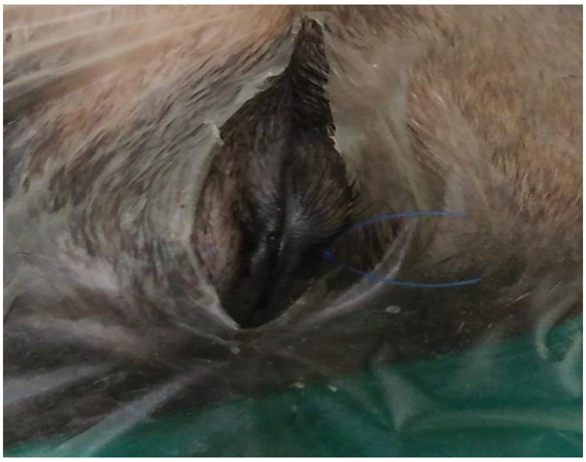


Fig. 62: Day 0 Intra-operative photograph showing closure of eyelids by partial tarsorrphy sutures

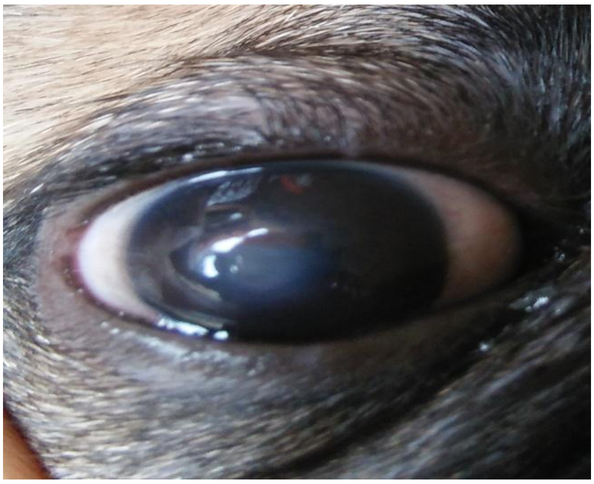


Fig. 63: Day 10 Post-operative photograph showing healed corneal ulcer with mild oedema

Fig. 64: Day 30 Post-operative photograph showing completely healed corneal ulcer with focal corneal oedema

GROUP II: AMNIOTIC MEMBRANE GRAFTING (ANIMAL 2)

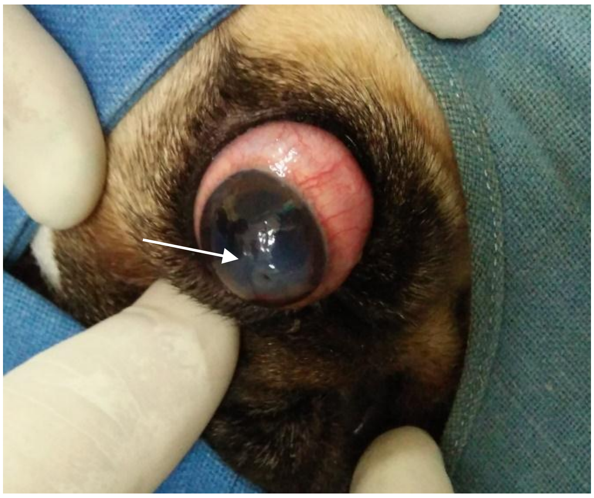


Fig. 65: Day 0 Intra-operative photograph showing exposure of central corneal ulcer

Fig. 66: Day 0 Intra-operative photograph showing application of amniotic membrane graft to recipient ulcer bed

Fig. 67: Day 0 Intra-operative photograph showing adhesion of amniotic membrane with cornea and careful closure of eyelids

Fig. 68: Day 0 Photograph showing closure of eyelids by partial tarsorrphy sutures

Fig. 69: Day 10 Post-operative photograph showing healed corneal ulcer with mild oedema

Fig. 70: Day 45 Post-operative photograph showing healed corneal ulcer with regain of normal corneal transparency

with range from ten to fifteen days in the study. Vongsakul *et al* (2009) reported mean epithelisation time as 7.33±0.21 days with canine amniotic membrane in conjunction with third eyelid flap for created corneal ulcers. However, Kruse *et al* (1999) observed mean epithelisation time and amniotic membrane dissolution time was four weeks and twelve weeks respectively following use of multilayer cryopreserved human amniotic membrane. Success rate of technique was evaluated in terms of restoration of vision and resumption of normal corneal transparency. Vision was restored in all 4 eyes and normal corneal transparency was resumed on 45th post-operative day. Kalpravidh *et al* (2009) observed normal restoration of corneal transparency within 35th post transplantation day with canine amniotic membrane transplantation. All the animals in group II recovered well. A success rate of 100% was recorded following amniotic membrane transplantation technique in present study.

In amniotic membrane grafting complications like neovascularization and corneal scaring were not evident. However, mild corneal oedema and little scaring of cornea in one case were noticed. Corneal oedema however, markedly subsided on 30th post-operative day. These observations were supported by Vongsakul *et al* (2009) who reported rapid epithelialization of cornea without neovascularization and scarring of ulcer bed in created corneal ulcers. Similar findings were reported by Kalpravidh *et al* (2009) who found corneal epithelialization time as 14 days without any neovascularization and scarring of cornea following canine amniotic membrane transplantation. Vongsakul *et al* (2009) stated that suturing of amniotic membrane with cornea resulted in increase in inflammation and corneal opacity. In present study amniotic membrane transplantation was done without any suturing. Amniotic membrane was applied directly to cornea. This might be the possible reason behind the greater corneal transparency in the operated animals in this group. Furthermore, amniotic membrane also possesses anti-angiogenetic effect, anti-scarring effect and anti-inflammatory action which makes it more suitable occular graft (Gomes *et al* 2005). Amniotic membrane act as a barrier against polymorphonuclear cell infiltration therefore, it significantly reduced the inflammatory mediators that otherwise invade the corneal stroma (Park and Tseng, 2000) thus favouring in alleviating the inflammatory response, neovascularization and anti-scarring effect (Hao *et al* 2000).

Intra-operatively establishment of adhesion between stromal side of amniotic membrane and epithelium of cornea was commonest complication. To overcome this complication amniotic membrane transplantation was done only in centrally located ulcers. However, it was observed that if amniotic membrane somehow failed to get complete adhesion and folded due to eyeball rotation, it will be on centre of eye where ulcer was located.

Complications in terms of neovascularization and corneal scarring were minimum in amniotic membrane grafting. Corneal oedema (2), corneal scarring (1) and corneal pigmentation were the complication observed in group-II. Ollivier *et al* (2006) proposed that eyes receiving amniotic membrane grafts possess minimal degree of corneal scarring and regained a greater transparency of cornea.

Table16: Complications recorded in animals treated with amniotic membrane grafting

Complication	Graft rejection	Corneal oedema	Corneal scaring	Pigmentation
Observations	No allograft rejection seen.	Corneal oedema was observed only at ulcer bed.	Corneal scarring was observed in one animal.	Pigmentation was observed in one eye. Pigmentation was subsided markedly upto 90th post-operative day.
No. of eyes affected	None	2	1	1

In present study none of allograft rejection was reported because of the fact that amniotic membrane was consist of avascular and non-immunogenic material. This finding was supported by the earlier study of Tseng *et al* (1999) who found that preserved amniotic membrane was devoid of any live cells and thus it does not initiate the immune reaction.

Group III- Surgical debridement

Debridement or epithelial debridement was surgical technique performed to remove loose necrotic corneal epithelium and to hasten healing of corneal ulcers. In Group-III eleven animals underwent debridement procedure under topical local propairacaine anaesthesia. Six eyes were debrided with beaver blade no. #64 and five

GROUP III-A: DEBRIDEMENT WITH BEAVER BLADE (ANIMAL 1)

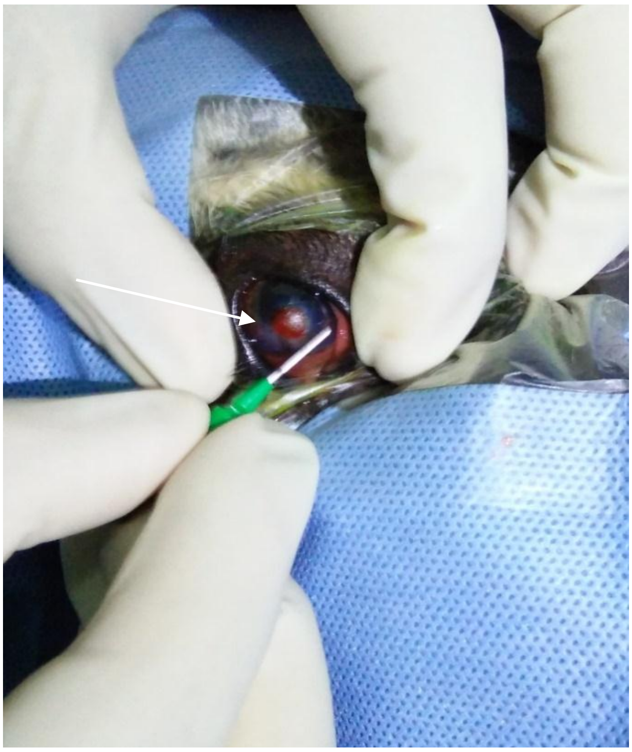


Fig. 71: Day 0 Intra-operative photograph showing debridement of dorso-nasal corneal ulcer with beaver blade

Fig. 72: Day 10 Post-operative photograph showing healed corneal ulcer with mild scarring and odema at ulcer bed

Fig. 73: Day 15 Post-operative photograph showing healed corneal ulcer with regain of corneal transparency

Fig. 74: Day 30 Post-operative photograph showing completely healed corneal ulcer with scar at ulcer bed

GROUP III-A: DEBRIDEMENT WITH BEAVER BLADE (ANIMAL 2)

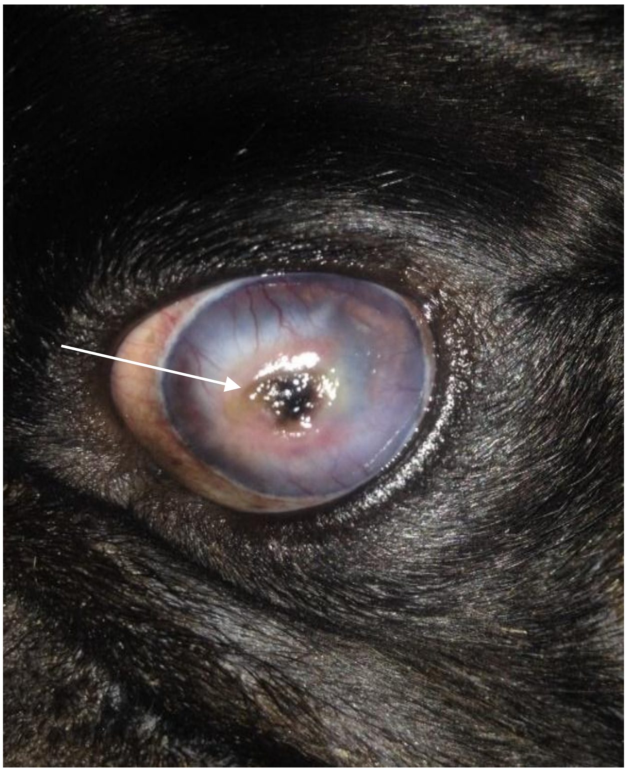


Fig. 75: Day 0 Pre-operative photograph showing central corneal sequestrum seen as area of central necrosis with brown discolorations

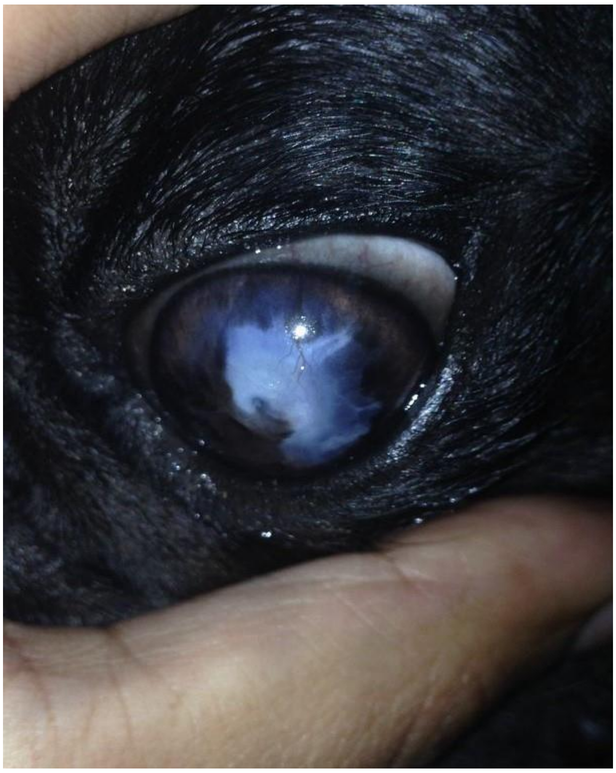


Fig. 76: Day 10 Post-operative photograph showing healed corneal ulcer with moderate scarring and odema at ulcer bed

Fig. 77: Day 30 Post-operative photograph showing healed corneal ulcer with mild scarring and pigmentation of cornea

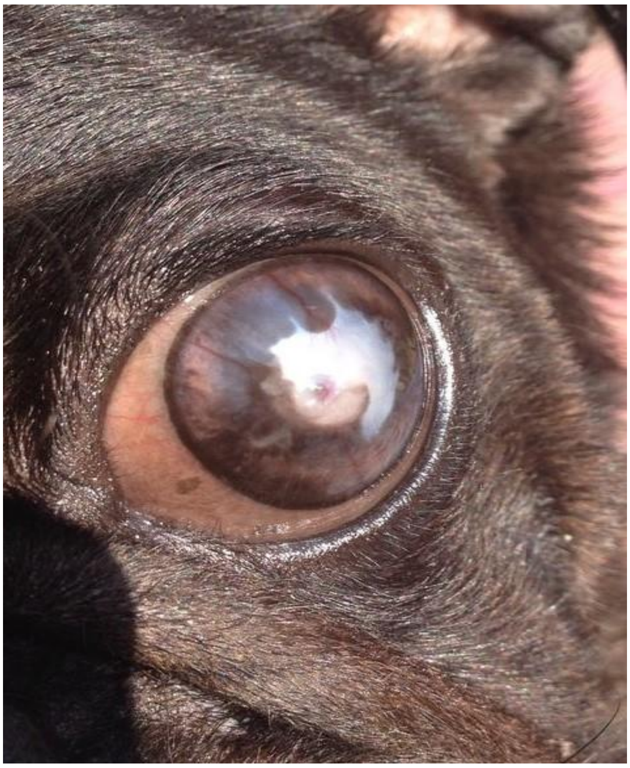


Fig. 78: Day 45 Post-operative photograph showing healed corneal ulcer with mild scarring and neovascularization of cornea

eyes were debrided with sterile cotton swab. After procedure all eyes were left open without any tarsorrhphy suturing. Eyes were examined on 10th post-operative day for outcome.

Group III-A— Debridement with beaver blade no. #64

Six eyes in six animals were debrided with the help of commercially available beaver blade no. #64. Eyes were left open after the procedure. All six eyes were healed within a mean±SE of 14.16±4.91 days (10 to 20 days). Four eyes out of six eyes, resumed functional vision. Overall success rate of technique was 66.67%. Out of two eyes that failed to resume vision, one had the severe keratitis and second having corneal sequestrum preoperatively. Eye having severe keratitis showed satisfactory healed corneal ulcer within 20 days of debridement procedure. Corneal sequestrum wound was healed completely with permanent scar and marked pigmentation followed by exophthalmos of same eye. It was more pronounced up to 40th post-operative day. Condition improved following topical steroid application. Moderate degree of corneal opacity and scarring was evident till last evaluation. In these two animals only the cosmetic appearance of eyes was optimized but functional vision was not restored till last examination upto 120 days following procedure. Corneal oedema was observed in all eyes following debridment. However, oedema was markedly subsided on 30th post-operative day. The level of corneal scaring was acceptable in all cases and it did not affect the menace reflex till last evaluation. One eye failed to heal on first debridement application, therefore second debridement procedure was performed after 7 days the first procedure. Moore (2003) stated that corneal ulcers that failed to heal on single application of debridement required repeated debridement application on 7-10 days apart.

Ulcer healed effectively with minimal corneal oedema and corneal scar within time period of 14.16±4.9 days. It was comparable to the healing time with that of grid keratotomy (14.0±5.5 days) and superficial keratectomy (9.3±3.9 days) as reported by (Stanley *et al* 1998). However, in view of (anssens (2007), Stanley *et al* (1998) and Moore (2003) although grid keratotomy and superficial keratectomy offers corneal ulcer healing within short time period, but these procedures required general anaesthesia, a greater degree of expertise and has a high cost of the procedure. Conversely, debridement with beaver blade was performed under topical local anaesthesia, a low cost procedure. It offers a good alternative to routine grid keratotomy and superficial keratectomy procedures. Furthermore, as the eyes were left open after procedure, therefore the progression of corneal ulcer was best monitored.

Table 17: Complications recorded following debridement with beaver blade (Group III-A)

Complication	**No. of treatments**	**Corneal oedema**	**Corneal scaring**	**Pigmentation**
Observations	Corneal ulcer did not heal in one animal. Second debridement procedure was performed after 7th day of first application.	Corneal oedema was observed in all animals. Oedema subsided markedly on 30th post-operative day.	Corneal scarring was observed in one animals having corneal sequestrum It was observed up to last evaluation.	Excessive pigmentation was seen in one animal having corneal sequestrum.
No. of eyes affected	1	3	1	1

Group III-B— Debridement with sterile cotton swab

Five eyes of five animals were debrided with the help of dry, sterile, cotton swab. Three applications of debridement in circular motion were applied on ulcer to remove loose necrotic epithelium. Eyes were examined on 10th post-operative day. Three eyes showed satisfactory healing of corneal ulcer with moderate corneal oedema and minimum scaring at ulcer site. Two eyes that failed to heal on first application needed multiple debridement applications. One eye developed bacterial keratitis and therefore multiple debridement applications were not able to restore the functional vision. Second eye had the fungal keratitis on pre-operative presentation. Anti-fungal drug (Ketoconazole @ 5mg/kg body weight per week orally was administered for 5 weeks Other topical medications were given as routine. Delayed positive menace reflex was noticed in this animal but overall vision was restored satisfactory.

On single debridement application, ulcer healing with restoration of functional vision was reported in 60% animals. However, overall 80% success rate was reported

GROUP III-B: DEBRIDEMENT WITH STERILE COTTON SWAB (ANIMAL 1)

Fig. 79: Day 0 Pre-operative photograph showing melting corneal ulcer seen as green epithelial lips of cornea

Fig. 80: Day 5 Post-operative photograph showing healing of corneal ulcer with moderate corneal oedema

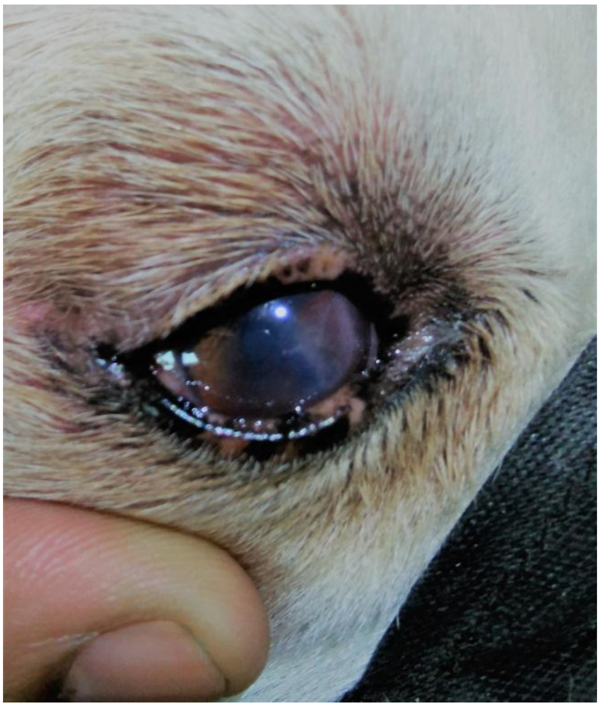


Fig. 81: Day 30 Post-operative photograph showing healed corneal ulcer with mild oedema

Fig. 82: Day 45 Post-operative photograph showing healed corneal ulcer with mild scarring at ulcer bed

GROUP III-B: DEBRIDEMENT WITH STERILE COTTON SWAB (ANIMAL 2)

Fig. 83: Day 0 Pre-operative photograph showing dorso-nasal corneal ulcer

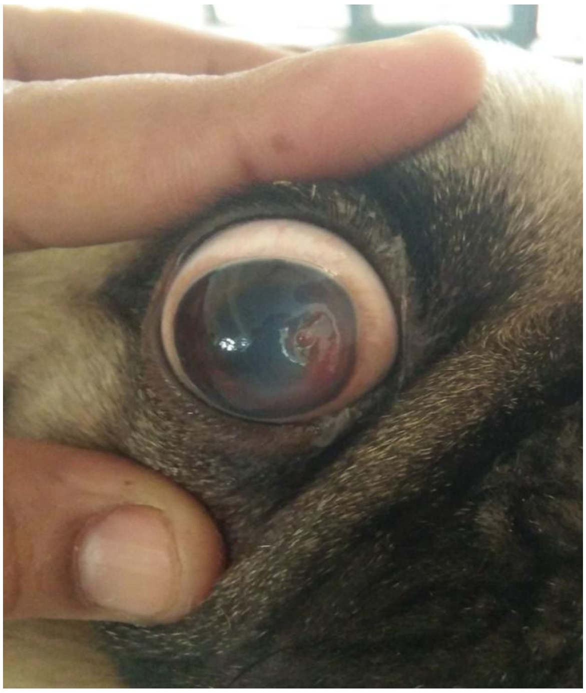


Fig. 84: Day 5 Post-operative photograph showing healing of corneal ulcer with mild oedema

Fig. 85: Day 10 Post-operative photograph showing healing of corneal ulcer with mild oedema

Fig. 86: Day 30 Post-operative photograph showing healed corneal ulcer with mild oedema

with this technique. These findings were supported by the observations of Stanley *et al* (1998) who reported success rate of 84% in corneal erosions managed by debridement with dry sterile cotton swab. Moore (2003) reported success rate of 63% on single application of debridement with dry sterile cotton swab. He reported overall success rate of 84% following repeated application of debridement. Minimum corneal oedema was observed till last evaluation in this group. This could be due to least traumatic injury to the corneal epithelium by cotton swab. One eye having melting ulcer or indolent ulcer developed greater extent of corneal scarring without neovascularization up to the last evaluation. Janssens (2007) stated that debridement with cotton swab applicator was the first choice of treatment for indolent ulcers along with the use of topical corticosteroids. Jones *et al* (2007) stated that where corneal lesions failed to heal after multiple debridement application, combined application of debridement and grid keratotomy was required. Combined use of epithelial debridement followed by grid keratotomy significantly decreases healing times for indolent ulcers. However, in present study no grid keratotomy was performed in animals where corneal ulcers were failed to heal on multiple debridement procedures.

Epithelium debridement or epithelium cauterization was useful to hasten corneal healing for indolent ulcers. Hvenegaard *et al* (2011) reported 100% healing rate in indolent ulcers within 30 days with epithelium debridement. Grid keratotomy was considered as safe and effective surgical treatment option for superficial indolent ulcers refractory to topical antibiotic treatment (Sarbani and Himangshu 2011; Singh *et al* 2014). Epithelial debridement should be the first line of treatment for the non-healing corneal ulcers. After the failure of this technique, others techniques like grid keratotomy and superficial keratectomy should be performed. However, Michau *et al* (2003) reported that single application of epithelial debridement significantly shortened the healing time period for indolent ulcers in horses as compared to grid keratotomy or superficial keratectomy. Croix *et al* (2001) found mean corneal ulcer healing time was 5 weeks with epithelium debridement for non-healing corneal ulcers.

Corneal oedema and corneal scarring were minimal in animals subjected to debridement with dry sterile cotton swab. It may be due to least trauma to corneal tissue.

Table 18: Complications recorded in animals treated by cotton swab debridement (Group III-B)

Complication	**Keratitis**	**Corneal oedema**	**Corneal scaring**	**Pigmentation**
Observation	One eye developed bacterial keratitis and second eye had fungal keratitis at presentation of case.	Corneal oedema was noticed on 10th day of procedure. Oedema Subsided on 30th post-operative day.	Corneal scarring was seen only at ulcer bed. Lighting of scarring with time was seen	Pigmentation was observed in 3 eye
No. of eyes affected	2	2	2	3

CHAPTER V

SUMMARY AND CONCLUSION

Study was included 28 eyes of 27 dogs with the history and clinical symptoms of ulcerative keratitis or corneal ulceration presented to the Department of Veterinary Surgery and Radiology, GADVASU, Ludhiana from the period of March 2016 to May 2017. All animals undergoing surgical treatment were selected after complete ophthalmological, bacteriological and corneal impression smear examination. Presented animals were randomly divided into three groups based on the surgical technique adopted: Group I (n=13) bulbar conjunctival grafting, Group II (n=4) amniotic membrane grafting and Group III (n=11) surgical debridement. Group I was divided into two sub-groups i.e. Group I A (n=7) in which conjunctival pedicle grafting was performed and Group I B (n=6) in which conjunctival bridge grafting was performed. Group III (n=11) was sub divided into two groups i.e. Group III in which debridement with beaver blade no.# 64 was done and . Group III B (n=5) in which debridement with sterile cotton swab was done.

Age wise distribution of corneal ulceration showed highest incidence (92.60%) in young age group (1-3 years). Male animals (66.67%) were more prone to corneal ulceration in study. Brachycephalic breed (pug) was the predominantly affected dog breed with corneal ulceration. Traumatic injuries to anterior epithelial (53.57%), keratoconjunctivitis sicca (28.57%), bacterial inflammation of cornea (14.28%) and melachezzia infection (3.57%) were most predisposing causes for the pathogenesis of corneal ulcers. Location based identification of corneal ulceration showed high numbers of centrally located corneal ulcers (60.61%).

All animals were subjected to basic neuro ophthalmic reflexes on the basis of outcome of basic visual functioning tests like menace reflex, pupillary light reflex, palpebral reflex and cotton ball test. Specific diagnostic tests like Fluorescein dye test and Schirmer's tear test were performed for confirmatory diagnosis of corneal ulceration.

Corneal swabs samples were the most common method for identification and isolation of prevalent ocular microbes. Main isolated micro flora includes *Staphylococcus spp. (71%)*, *Bacillus spp. (19%)* and *Streptococcus spp. (10%).*

Culture sensitivity profile was carried out to determine the antibiotic sensitivity. Antibiotics viz. Gentamicin, Tobramycin, Neomycin, Gatifloxacin, Ofloxacin, Ciprofloxacin, Amoxycilin, Ampicillin were found most sensitive antibiotics in the study. Cytological examination of corneal impression smears revealed predominance of neutrophils and squamous epithelial cells. On the basis of presence or absence of any inflammatory cell, the cytology was categorized into inflammatory (68%) and non-inflammatory (32%) type.

All the cases of corneal ulcers were instilled topical intra ocular administration 0.3% tobramycin topical antibiotics drops, topical non-steroidal anti-inflammatory drugs (NSAID) ketorolac, and topical artificial tear drops carboxy methyl cellulose sodium starting from the day of presentation.

Anaesthetic protocol used for conjunctival grafting and amniotic membrane grafting included premedication with combination of Glycopyrrolate @ 0.01mg/kg, Acepromazine maleate @ 0.05mg/kg and Butorphenol @ 0.2mg/kg body weight by intramuscular route. Induction was performed with Diazepam @ 0.25 mg/kg of body weight and Ketamine @ 5 mg/kg of body weight. Anaesthesia was maintained with 1 to 2% Isoflurane. Local anaesthetic 0.5% proparacaine was used topically in animals subjected to surgical debridement (Group III).

Surgical management of corneal ulceration was carried out in 28 eyes of 27 animals. Animals were positioned in lateral recumbency with affected eye placed dorsally, followed by draping of animal with commercially available eye drape.

In group I-A bulbar pedicle conjunctival grafting was performed in seven eyes, followed by partial temporary tarsorrhphy. Clinical examination showed six eyes (n=6/7; 85 %) having intact graft to ulcer bed. In one eye (15%) self-mutilated graft dehiscence on next day of procedure was observed. Marked neovascularization of conjunctival graft was noticed in all eyes. However, no blood vessel was seen invading the normal cornea. Corneal oedema was evident in all eyes and was observed around the periphery of needle bite. All animals were evaluated on 30th post-operative day for stage of healing, extent of corneal oedema and level of neovascularization. It was observed that all ulcers were healed completely with marked subsided corneal oedema and satisfactory conjunctival graft neovascularization. High success rate (85.71%) was achieved in animals treated with bulbar conjunctival pedicle graft. After 45th post-operative days graft trimming was done. Scar was present at ulcer bed up to last evaluation. were noted as graft dehiscence, corneal oedema, corneal neovascularization, mild keratitis, scar at ulcer bed and corneal pigmentation were the post-operative complications in group I-A.

Bulbar bridge conjunctival grafting accompanied by partial temporary tarsorrhphy suturing was performed in six eyes. Three eyes (50%) had intact graft to ulcer bed. In three eyes (50%) graft mutilation was observed on 4th, 7th and 14th post-operative day respectively. Conjunctival graft neovascularization was evident on 10th post-operative day. Due to graft dehiscence in one eye bridge conjunctival graft was converted to conjunctival pedicle graft on 4th post-operative day. In one eye graft dehiscence was observed on 14th post-operative day. However, graft was still covering the ulcer bed, though it was torn from the base on both sides. Four eyes (66.67%) resumed to vision with acceptable scar at ulcer bed till last evaluation. Two eyes that failed to restore vision were because of the descemetocele nature of the lesion. However, cosmetic appearance of both eyes was preserved. In one case, bridge or bipedicle grafting yielded the thick granulating tissue, covering almost 70% of the cornea that hampered the vision. Graft dehiscence, corneal oedema, mild keratitis, scar at ulcer bed and corneal pigmentation were the most significant post-operative complications in animals operated by bridge conjunctival grafting technique.

Dry human amniotic membrane overlay graft was applied in four eyes. Two layered human amniotic membrane graft was placed over ulcer without suturing or any tissue adhesive. It was followed by partial temporary tarsorrhphy. Human amniotic membrane transplantation was applied as double layer membrane transplant. All animals treated by overlay amniotic membrane graft showed healed corneal ulcer within 11.25±2.50 days. Success rate of technique was evaluated in terms of restoration of vision and resumption of normal corneal transparency. Vision was restored in all 4 eyes and normal corneal transparency was resumed on 45th post-operative day. A 100% success rate was recorded following amniotic membrane transplantation technique. Complications in terms of neovascularization and corneal scarring were minimum in amniotic membrane grafting group as compared to bulbar conjunctival grafting. None of allograft rejection was reported because of the fact that amniotic membrane consisted of avascular and non-immunogenic material.

In group III-A six eyes of six animals were debrided with the help of commercially available beaver blade no. #64. Eyes were left open after the procedure. All six eyes were healed within period of 14.16±4.91 days (10 to 20 days). Four eyes resumed functional vision with overall success rate of technique 66.67%. Two animals having severe keratitis and corneal sequestrum preoperative failed to resume vision. In these two animals only the cosmetic appearance of eyes was optimized but functional vision was not restored till last examination upto 120 days following procedure. Moderate degree of corneal opacity and scarring was observed till last evaluation. Corneal oedema was observed in all eyes following debridment. However, oedema was markedly subsided on 30th post-operative day. The level of corneal scaring was acceptable in all cases and it did not affect the menace reflex till last evaluation. One eye failed to heal after first debridement procedure, therefore second debridement was attempted 7 days after first procedure. Debridement with beaver blade was performed under topical local anaesthesia which is a low cost procedure and it offers a good alternative to routine grid keratotomy and superficial keratectomy procedures. Furthermore, as the eyes were left open after procedure and therefore progression of ulcer was best monitored.

In group III-B five eyes of five animals were debrided with the help of dry, sterile, cotton swab. Three applications of debridement in circular motion were applied on ulcer to remove loose necrotic epithelium. Three eyes showed satisfactory healing of corneal ulcer with moderate corneal oedema and minimum scaring. Two eyes needed multiple debridement applications that failed to heal after first application. Overall success rate of 80% was reported by this technique. One eye that had the melting ulcer (indolent ulcer) developed greater extent of corneal scarring without any neovascularization observed up to last evaluation. Corneal oedema and corneal scarring were minimal in animals subjected to debridement with dry sterile cotton swab.

Based on the above results following conclusions were made from the present study:

- * Corneal ulceration was a common ophthalmic condition of young male dogs. Pug breed dogs were more vulnerable to corneal ulceration.
- * Staphylococcus spp. was the most prevalent ocular microbe isolated from the corneal swabs samples and Tobramycin and neomycin were the most sensitive topical antibiotics.
- * Cytological findings reveals that majority of cases were of inflammatory type with predominance of neutrophils in conjunction with clumps with bacteria.
- * Human amniotic membrane transplantation technique offered rapid corneal epithelisation with greater transparency and minimal corneal scarring. High cost of amniotic membrane graft though limits its routine use in veterinary ophthalmology
- * Conjunctival pedicle graft was found better surgical technique than bridge conjunctival graft technique as it has minimal post-operative complication and it was an economical procedure.
- * Debridement with beaver blade technique offered complete corneal epithelisation within short time period than debridement with sterile cotton swab technique.
- * Human amniotic membrane transplantation technique was better surgical management technique than conjunctival pedicle grafting, bridge conjunctival grafting, debridement with beaver blade and debridement with sterile cotton swab technique.

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