EFFICACY OF COLLAGEN SHEET FOR THE MANAGEMENT OF CORNEAL ULCERS IN DOGS

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DECLARATION

I hereby declare that this thesis, entitled **"EFFICACY OF COLLAGEN SHEET FOR THE MANAGEMENT OF CORNEAL ULCERS IN DOGS"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Certified that this thesis, entitled **"EFFICACY OF COLLAGEN SHEET FOR THE MANAGEMENT OF CORNEAL ULCERS IN DOGS"** is a record of research work done independently by **CHINCHU JOSE**, under my guidance and supervision and it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Chinchu Jose

Dedicated to my family

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Introduction

1. INTRODUCTION

Corneal ulcers are the most common ocular emergencies in dogs. A deep ulcer involves the loss of corneal stroma in addition to the loss of epithelium. As the condition persists, the ulcer invades the tissue completely and leads to staphyloma.

Lagophthalmic breeds are frequently susceptible to central corneal ulcers; ulcers in them can progress and deepen rapidly (Mandell and Holt, 2005). Deep and/or progressive corneal ulceration are the most frustrating of ocular problems, which may lead to loss of vision. The brachycephalic breeds like Chinese Pugs are more prone to corneal injury due to their inherited corneal insufficiency and a lack of protective eye consciousness. In addition, the increased intraocular pressure in them resulted the weakening of the central cornea.

Ulcerative keratitis is one of the most important manifestations of keratitis. Uncomplicated superficial ulcers usually heal readily, with minimal scar formation. Complicated deep ulcers may lead to impaired vision because of corneal scarring or, when corneal perforation occurs, to anterior synechia formation. Severe ulcerative keratitis may lead to loss of the eye because of endophthalmitis, glaucoma, phthisis bulbi, or some combination of these (Gilger *et al*., 2008). In most cases, if prompt and suitable treatment is provided, could achieve reasonable healing even if perforation has occurred.

Every corneal lesion along with the proper history collection should undergo a detailed ophthalmic investigation such as Schirmer's tear test, fluorescein dye test, measurement of intraocular pressure, pupillary light reflexes, ophthalmoscopy and assessment of the visual status which are the essential components of any ophthalmic examination (Moore, 2001).

Simple corneal ulcers are superficial with acute onset and are usually painful because of the sensory nerve endings in the anterior epithelium. If untreated it may lead to deep ulcers or even up to corneal perforation (Bedford, 1987). Corneal perforations are always an ocular emergency as it may lead to the loss of aqueous humor, severe pain, intraocular infections and finally the loss of vision.

The usual treatment for the corneal lesions in dogs includes topical medications, scarification or suturing along with routine medicinal application. Mostly the simple superficial corneal ulcers heal in a week time with medical care but the complicated cases were found to take longer course to heal. Actually the surgical intervention was found to reduce the healing time. However, to achieve the clarity of the cornea, it takes prolonged time.

The healing process of the corneal defect is mainly depending on the severity of the condition as well as the depth or the thickness to which the defect is involved. Surgical interventions for the corneal lesions includes debridement, phenol cauterization, superficial keratectomy, punctate keratectomy, grid keratectomy and different bandaging procedures like contact lenses, collagen shields, cyanoacrylate tissue adhesives, temporary tarsorrhaphy, third eyelid flap and conjunctival flaps (Wilkie and Whittaker, 1997).

The usefulness of equine pericardium (Barros *et al*., 1999), porcine small intestinal submucosa (Lewin, 1999) and amniotic membrane (Barros *et al*., 2005; Kim *et al*., 2009 and Plummer, 2009) for the repair of full thickness defects of cornea in dogs had been reported.

Collagen was found to reduce the healing period of corneal ulcer. Bovine intestinal submucosa as collagen sheet is an easily available collagen source and can easily manipulate for surgical techniques. Higher incidence of corneal ulcers in ophthalmic cases is probably due to the tremendous increase in the population of brachycephalic breeds like Chinese Pugs.

Collagen of different origin has been used for the management of corneal lesions in dogs, but it requires further investigation to study its efficacy, suitability and probable complications associated with its use. Hence the study was undertaken with the objective to evaluate the efficacy of collagen sheets in the healing of corneal ulcers in dogs.

Review of Literature

2. REVIEW OF LITERATURE

2.1. ANATOMY OF THE CANINE CORNEA

Shively and Epling (1970) observed that the canine cornea consists of tear film, corneal epithelium and its basement membrane, stroma, Descemet's membrane and endothelium. A complete Bowman's membrane was absent in canines.

Startup (1984) described that the cornea occupies the anterior 1/6th of the globe and radius of curvature is slightly greater than the remainder of the globe. Histologically consisting of four layers viz. corneal epithelium, corneal stroma, Descemet's membrane and endothelium.

The cornea is a transparent structure, which comprises one-fifth of the fibrous tunic of the eye. Its transparency depends upon a number of features like its avascularity, dehydrated state, unmyelinated nerves, lack of pigmentation, non-keratinised epithelium, well organised lamellae within the corneal stroma and the interval between collagen fibrils is constant (Renwick, 1996).

The canine cornea measures 12 to 16 mm vertically and 13 to 17 mm horizontally and the axial cornea is thinner than the periphery (Wilkie and Whittaker, 1997).

Marfurt *et al.* (2001) described that the cornea is one of the most richly innervated tissues in the body, receiving dense sensory innervation from the trigeminal nerve and sympathetic innervation from the superior cervical ganglion. Corneal sensory and sympathetic nerves exert important neuromodulatory effects on corneal epithelial physiology, including, regulation of ion transport; cell proliferation, differentiation, adhesion, and migration; and wound healing.

Miller (2001) described that the anterior epithelium has a typical stratified squamous epithelial architecture composed of superficial flattened cells, 2 to 3 layers of wing or intermediate cells, and a basal layer of columnar cells. The stroma accounts for about 90% of the cornea consisting of collagen, glycosaminoglycans and water. Descemet's membrane is the basement membrane of the endothelium and is elastic and resistant to enzymes that denature the stroma. The endothelium is a monolayer of hexagonally shaped cells on the posterior surface of the cornea.

Herring (2003) explained that the keratocytes *i.e*. the specialized fibroblasts present within the corneal stroma is responsible for the maintenance of stromal collagen. Lamellae are formed by parallel bundles of collagen fibrils separated by ground substance. The collagen fibrils are of uniform diameter and are uniformly spaced within the lamellae. Though all fibrils within a lamella are parallel to each other, the fibrils of adjacent lamellae run at an angle to each other.

The normal cornea is a clear transparent structure composed of several layers: epithelium, subepithelial basement membrane, stroma, Descemet’s membrane and endothelium. Structural adhesion of the epithelial layer to the underlying anterior stroma, provided by hemidesmosomes and anchoring fibrils that are firmly attached between the basal epithelial cells and the subepithelial basement membrane (Janssens, 2007).

Kafarnik *et al*. (2007) reported that the comparison of the confocal images of normal cornea in dogs and cats did not reveal any morphologic differences.

According to Andrew (2008) cornea of dogs composes of five layers: an outer stratified squamous epithelium, epithelial besement membrane, stroma, Descemet’s membrane, and the inner endothelium. The main tissue components are collagen (types I, III, IV, V, VI, VII, VIII, and XII) and glycans (laminin,

fibronectin, hyaluronans, heparin sulphate, chondroitan 6-sulfate, chondroitan 4-sulfate, dermatan sulphate, tenascin, and P component).

Hoffman *et al*. (2009) pointed out that the corneal endothelium is a metabolic structure characterized by a single layer of flattened cells lining the inner cornea in a hexagonal interdigitating arrangement. Pronounced pleomorphism has been observed in rabbit, cat, and older dog. Young dogs have an endothelial cell density of approximately 2900 cells/mm^2, which gradually decreases by 50% or more with age.

Nagayasu *et al*. (2009) observed that the site-dependent difference in the cornea is closely associated with function and maintenance of the unique shape of the eyeball. The central portion of the cornea is abundant in collagen fibrils of small diameter, which prevent slippage between collagen fibrils and thereby impart their elasticity. These fibrils also buffer the direct impact of intraocular pressure on the central portion of the cornea.

2.2. PHYSIOLOGY OF THE CANINE CORNEA

Startup (1984) observed that the cornea must be transparent inorder to transmit light freely and is dependent on its avascularity, its uniform structure and its deturgescent stage. The avascularity will reduce the surface temperature by 0.5-1^o C and breeds with large corneal surface are likely to show less resistance to corneal infection and ulceration because of lowered temperature and sensitivity.

According to Gum (1991) the energy requirement of the cornea in the form of ATP was furnished mainly by the aqueous humor and is mainly utilized to maintain the state of dehydration. Under stress, the glycogen storage in the epithelium acts as the energy source and therefore if these stores become depleted, normal healing of the epithelium and cellular locomotion over the surface will be inhibited.

The transparent, avascular cornea serves to transmit and refract light and has a protective function for the intraocular contents. The corneal epithelium is completely replaced every 7 to 14 days. Disruption of either the epithelium or endothelium will result in corneal hydration i.e. corneal edema (Wilkie and Whittaker, 1997).

Blocker and Woerdt (2001) pointed out that brachycephalic dog breeds have decreased corneal sensitivity compared to mesaticephalic and dolichocephalic breeds.

Miller (2001) reported that superficial corneal ulcers tend to cause pain by exposing nerve endings within the inter-mediate cell layer. The interstitial compounds of stroma are hydrophilic, readily attracting water from the tear film and aqueous and when a damage to the epithelium or endothelium occurs, it imbibe fluid into the stroma, separating the collagen layers and giving the cornea a bluish discoloration.

Transparency of the cornea is a result of minimal light scattering that appears to be facilitated by the highly organized and avascular nature of the cornea's components. The cornea is the source of over two thirds of the total refractive power of the eye's optical system because of its curvature and physioanatomical features (Xie *et al.*, 2002).

Herring (2003) described that the cornea has a mechanical function to maintain the physical integrity of the eye along with sclera and the optical function to provide clear passage for light transmission and to retract or bend the light thus assisting in focusing. The corneal stroma is hydrophilic, but is maintained in a state of relative dehydration by active pumping mechanisms of the corneal endothelium and barrier function of the anterior corneal epithelium.

Morreale (2003) noted that the transparency of the cornea is maintained by the smooth, non-keratinized, squamous epithelium, which further is enhanced by the precorneal tear film, the lack of corneal vascularization or pigmentation,

the size and regular arrangement of collagen fibrils that make up the corneal stroma, and the relative dehydration of cornea.

The anatomical integrity of the cornea and the associated immune status of the host is the defence against bacterial invasion. Tears also defend the cornea by the presence of lysozyme, lactoferrin, etc. and by the constant movement. The active sloughing of the corneal epithelial cells (every 5 to 7 days) and Descemet's membrane inhibits invasion of microorganisms (Ollivier, 2003).

The endothelium has an active pump mechanism that regulates the ion composition of the corneal stroma, thereby maintaining a constant osmotic pressure and thus controlling hydration of the cornea. This mechanism maintains a constant thickness and transparency of the cornea (Rodrigues *et al.*, 2006).

Andrew (2008) described that the limited immune response of cornea is due to the lack of lymphatics and blood vessels and the cornea especially the central cornea was considered as an immune privileged tissue.

Hoffman *et al.* (2009) pointed out that Na+/K+ ATPase pumps are located principally within the endothelium to maintain deturgescence and corneal clarity. If the endothelium is not functioning optimally, corneal oedema and progressive corneal disease may result.

2.3. CORNEAL HEALING

Befanis *et al.* (1981) studied re-endothelization occurred by both mitotic division and enlargement and migration of remaining undamaged cells. Reestablishment of a continuous intact endothelial cell monolayer occurred at six weeks and the corneal endothelium of the young adult canine has marked regenerative potential.

Panjwani *et al*. (1995) opined that after injury to the cornea, initial healing of the wound takes place by the migration of adjacent cells to the injured area.

Chandrashekar *et al.* (1997) observed the fibroblastic changes in the reconnected stroma in corneal grafting and fibrous tissues project into the anterior chamber through the existed gap between Descemet's membrane and the epithelial layer and stroma were reconstituted by fourth week and completed formation and union by sixth week.

Willeford *et al.* (1998) described the healing of a corneal defect which started with the migration of epithelial cells. Fibronectin, fibrin, laminin and other extracellular matrix proteins form a matrix creating a scaffold for the migrating epithelial cells. During this migration, these epithelial cells release plasminogen activator in the presence of fibronectin and fibrin. Conversion of plasminogen to plasmin, result the detachment of newly formed scaffold. This enables the epithelial cells to form permanent anchoring fibrils to the underlying basement membrane. This cascade continues until the defect was covered with firmly attached epithelial cells.

Whitley (2000) opined that uncomplicated superficial ulcers heal with minimal scar formation but complicated deep ulcers may lead to impaired vision because of corneal scarring or, when corneal perforation occurs, to anterior synechia formation. Healing of refractory ulcers may take weeks to months, and recurrence is more because of the de-epithelialized corneal predisposition to the bacterial infection.

According to Featherstone *et al*. (2001) the cornea owes its unique transparency to the precise organization of predominantly collagen type 1 fibrils, which are oriented in a parallel manner. During normal stromal healing, type III collagen laid down and arranged less regularly, thus resulted in corneal scarring and possible visual impairment.

Miller (2001) observed that when the stroma is damaged, keratocytes are altered to form fibroblasts, which produce collagen to fill the defect, but the random collagen deposition produces scar. When stromal architecture is

disrupted, limbal blood vessels may progress toward the wound. Once vessels have invaded the cornea, they will always remain, although they may not always carry blood after wound healing.

According to Xie *et al*. (2002) the surgical procedures that do not penetrate stroma had a faster wound healing response and rapid visual recovery, since the slow regeneration of the stroma compared to the epithelium.

Herring (2003) opined that the inadequate of nutrients and oxygen supplied by the tear film could be detrimental to the corneal health and post surgical healing.

Moore (2003) stated that the corneal epithelial cells migrate into the site of wound within the first hour of corneal injury and a fibrin and fibronectin mesh will be deposited which will help the adhesion of epithelium to the stroma. The plasminogen activator released from the migrating cells convert the plasminogen into plasmin, which cleave old epithelial attachments and allows migration across the wound. The adhesion between the epithelial cells and stroma completed within one week.

Bentley and Murphy (2004) described that the corneal wound healing includes epithelial wound healing, stromal wound healing and endothelial wound healing. Corneal wound healing was described as a complex process, which involves the interplay between cellular elements of the cornea, cytoactive factors, constituents of extracellular matrix and biochemical forces.

Brooks and Ollivier (2004) noted that the response to the corneal injury mediated by leukocytes, fibroblasts, and vascular endothelial cells and includes spatiotemporal phases of inflammation, angiogenesis, re-epithelialization, granulation tissue formation, and extracellular matrix deposition. Matrix metalloproteinases are mediators of enzymatic activity during corneal repair and collagen remodelling.

Bentley (2005) stated that after a lag phase following corneal injury, epithelial sheets migrates centripetally, dissembling the adhesion between the epithelial cells and basement membrane. After migration and proliferation of epithelial cells, adhesion complexes between epithelial cells and basement membrane are re-epithelialised. Delayed reassembly of adhesion complexes results when basement membrane was damaged.

Mandell and Holt (2005) stated that the uncomplicated superficial ulceration heal within 3 to 5 days, while the stromal ulcers in which more than one third of the corneal thickness is involved may take about 3 weeks to heal.

Rodrigues, *et al.* (2006) pointed that the endothelial cells were at risk of being damaged or lost from intraocular disease and have a low regenerative capacity and a low replication rate. Endothelial cell division was poor following trauma or disease and undergone hypertrophy to cover a denuded Descemet's membrane following damage caused by trauma or in response to a decrease in endothelial cell density (ECD) with advancing age.

According to Williams *et al.* (2008) corneal healing following surgery involves the coordination of numerous cellular processes such as cellular migration and proliferation, activation of keratocytes, and the expression of growth factors. The role of Type-IV collagen expression in corneal wound repair is being associated with corneal haze and visual acuity loss.

Carter (2009) described that the corneal epithelium was maintained by the combined process of basal epithelial cell proliferation, shedding of superficial epithelial cells, and renewal by the centripetal migration of basal epithelial cells originating from stem cells at the limbus. More rapid healing occurs when the basement membrane was intact and components of the adhesion complex could be reused. Stromal wound healing involves the transformation of keratocytes to fibroblasts, which proliferate to synthesize collagen and extracellular matrix (ECM) components. Stromal collagen became cross-linked and proteoglycan

synthesis occurred to result in gradual wound remodelling. Anterior displacement of the overlying epithelium occurs gradually by the new collagen fibres and lamellae, which often disorganized and result in scar formation.

Hoffman *et al*. (2009) observed that the adult canine endothelial cells do not respond to injury with active mitosis. Instead, they enlarge and migrate to form a monolayer.

2.4. CORNEAL ULCER

According to Renwick (1996) ulcerative disease may be subdivided into erosions which involve only the corneal epithelium and ulcers which additionally involve varying depths of the underlying corneal stroma.

Ollivier (2003) described Bacterial cornea1 diseases in dogs and cats were mostly represented by ulcerative keratitis and were very frequent. Because some of these cornea1 ulcers can be very severe, which progress rapidly and therefore was a possible cause of vision loss, it was important to diagnose them at an early stage and to adopt an appropriate treatment.

Corneal ulcer involves the loss of corneal stroma in addition to loss of epithelium and accompanies a variable degree of reflex uveitis (Mandell and Holt, 2005).

Bouhanna *et al*. (2008) opined that ulcerative keratitis in brachycephalic dog is usually recurrent and is located in a central or paracentral corneal position.

Kim *et al*. (2009) described ulcerative keratitis as one of the most common ocular problems leading to pain and vision loss in man and dogs. Immediate and effective treatment is important to maintain or restore vision and reduce pain. For these reasons, many surgical techniques have been introduced such as use of the conjunctival pedicle flap, small intestinal submucosa graft and amniotic membrane transplantation.

2.4.1. Incidence

2.4.1.1. Age

Murphy *et al*. (2001) described that the Spontaneous Chronic Corneal Epithelial Defects (SCCED) with no apparent underlying cause frequently encountered typically in middle aged to older dogs averaging 8 to 9 years of age. The average canine patient was middle aged to elderly.

Spontaneous chronic corneal epithelial defects in dogs typically found in middle-aged dogs of all breeds (Bentley, 2005).

According to Janssens (2007) indolent ulcers were usually seen in middle aged to older dogs.

2.4.1.2. Sex

Murphy *et al*. (2001) described that the clinical findings showed spontaneous chronic corneal epithelial defects in dogs which had no clear sex predilection.

Janssens (2007) stated that indolent ulcers have no sex predisposition in dogs.

2.4.1.3. Breeds

Bedford (1982) observed predisposition of corneal ulceration in Pekingese, corneal erosion in Boxer, keratitis pigmentosa in Pug and chronic superficial keratitis in German Shepherd Dogs. There was increased occurance of complete prolapse of the globe in brachycephalic breeds even with negligible amount of force, which may later be complicated by corneal desiccation and exposure keratitis.

Breeds like Pugs and Pekingese has inherited corneal insufficiency, poor corneal reflex and a lack of protective eye consciousness. These breeds were predisposed to nerve deficiencies especially on central corneal areas thus leading to neurotrophic ulcers. The chronic epithelial erosions were more pronounced in Boxer and Corgi (Startup, 1984).

Wolfer and Grahn (1994) stated that ulcerative keratitis is common among the brachycephalic breeds, as they are predisposed to corneal trauma due to globe prominence, lagophthalmos, and decreased corneal sensitivity. This may account for the frequency with which melting corneal ulcers seen in these breeds.

Murphy *et al*. (2001) observed predisposition of SCCED in breeds like Boxers, Golden Retrievers, and Keeshonds.

Lagophthalmic breeds were frequently susceptible to central corneal ulcers and deepen rapidly in them (Mandell and Holt, 2005).

Raji (2006) reported the higher incidence of corneal ulcers in Chinese pugs. Among them juveniles between 3.5 months to 24 months with an average age of 14.4 months were having maximum susceptibility.

Hendrix and Cox (2008) observed that the brachycephalic breeds have shallow orbits, excessive prominence of the globe, decreased corneal sensitivity, and reduced tear film stability. Many brachycephalic dogs also have lagophthalmos, trichiasis, and distichiasis.

Resmi (2008) reported that incidence of corneal lesions was more common in brachycephalic breeds like Pugs and the juveniles were mostly affected.

2.4.2. Classification

According to Renwick (1996) corneal ulcers were classified as superficial or deep depending on the amount of stromal tissue lost.

Wilkie and Whittaker (1997) classified corneal ulcers according to the depth, size, etiology, presence or absence of infection, and collagenase activity.

Whitley (2000) classified corneal ulcers by the depth of corneal involvement: as superficial, deep stromal, and descemetocele. Superficial corneal ulcers were further classified as uncomplicated, progressive, or refractory.

Miller (2001) developed a grading system to describe corneal ulcers. Grade 1 ulcers were superficial wounds that generally heal without complication and require routine therapy. Grade 2 ulcers were also known as persistent corneal ulcers, and do not responded to routine therapies and required surgical interventions. Grade 3 ulcers involved the corneal stroma but were not progressive. Grade 4 ulcers were progressive demonstrating destruction of surrounding corneal stroma. Grade 5 ulcers leads to perforation of cornea.

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

Corneal ulcers were classified by the depth of corneal involvement as superficial, deep stromal, descemetocele, and perforating ulcers. Superficial ulcers further divided into uncomplicated ulcers and persistent ulcers. Stromal ulcers are divided into progressive and non progressive types (Ollivier, 2003).

Gilger *et al*. (2008) classified ulcerative keratitis by the depth of corneal involvement as superficial corneal ulcer, deep stromal corneal ulcer, descemetocele and corneal perforation and by the underlying causes as bacterial, fungal, traumatic, immune mediated, indolent ulcers etc.

2.4.2.1. Superficial corneal ulcer

Superficial corneal ulcers commonly occur in our domestic species with signs like blepharospasm and photophobia, and often spontaneously resolve without treatment. Superficial ulcers, demonstrated by a positive fluorescein

stain, usually responded rapidly to antibiotic and clycloplegic therapy. Such patients should reevaluated within a week to assess healing (Miller, 2001).

Moore (2003) opined that superficial ulceration involved epithelium and basement membrane, with minimal or no involvement of corneal stroma.

According to Ollivier (2003), superficial ulcers were usually not infected and healed rapidly within a few days with minimal scar formation, but persistent ulcers healed slowly and showed tendency to recur.

Superficial corneal defects were relatively clear in the cornea and some were visible only with fluorescein dye. Uncomplicated ulcer should heal by re-epithelisation within three to five days (Mandell and Holt, 2005).

2.4.2.2. Indolent ulcer

According to Renwick (1996), recurrent epithelial erosions (REEs, indolent ulcer, boxer ulcer) were seen in any breed or crossbred dog but were classically described in the Boxer and Pembroke Corgi, predominantly in middle-aged and older dogs. They result from either abnormal basement membrane structure or reduced adherence of overlying epithelium, or the presence of pre-existent corneal oedema. Recurrent epithelial erosions may recur at any time in the same or the fellow eye.

Whitley (2000) stated that indolent ulcers recognized by the characteristic peripheral lip of undermined epithelium, which was not attached to the subjacent corneal stroma or epithelial basement membrane.

According to Moore (2003), indolent ulcers were the most common refractory ulcers in veterinary medicine and are persistent, superficial ulcerations with a non-adherent epithelial lip and without stromal involvement.

Superficial ulcers that do not heal in expected time schedule were referred as indolent, refractory, or nonhealing ulcers, and may be the result of an anterior stromal or epithelial basement membrane defect (Mandell and Holt, 2005).

Bentley (2005) described the clinical appearance of an indolent ulcer as superficial, non-infected erosion, surrounded by a sheet of non-adherent or loose epithelium. The epithelium may appear thickened, and fluorescein stain often leaks beneath the abnormal, non-attached epithelium, thus resulting in a less intense ring of staining around the exposed stroma. This condition occurs in all breeds of dogs, although boxers were over represented.

Ledbetter *et al*. (2006) enlisted the synonyms for spontaneous chronic corneal epithelial defects, as recurrent corneal erosion, persistent corneal erosion, refractory epithelial erosion, indolent ulcer, Boxer ulcer and rodent ulcer. They defined spontaneous chronic corneal epithelial defects (SCCEDs) as superficial epithelial defects that have no apparent inciting cause, do not involve the stroma, were bordered or partially covered with nonadherent epithelium, and failed to heal in a normal time period of one week.

Brunott *et al*. (2007) observed that in superficial indolent corneal ulcer, a rapid and simple healing process does not occur. This failure in repair of epithelium attributed to a failure to generate normal basement membrane. The characteristic features of superficial nonhealing corneal ulcers were a non-infected epithelial defect without stromal loss, and a positive fluorescein staining that was present for a minimum of 7 to 10 days, depending on its size.

Bouhanna *et al*. (2008) opined that refractory ulcer in dogs may attributed to primary or secondary to eyelash abnormalities like entropion, facial nerve paralysis, lagophthalmos, keratoconjunctivitis sicca, tear film mucin deficiencies, or neurotrophic keratitis.

Carter (2009) described recurrent epithelial erosions (REE) were characterized by a lack of adherence of the migrating corneal epithelial cells to the underlying stroma.

2.4.2.3. Deep stromal corneal ulcer

Bedford (1987) opined that marked corneal oedema and stromal vascularisation usually accompanies deep ulceration.

Superficial ulcers may turn into deep stromal ulcers because of infections, tear film and eyelid abnormalities, inappropriate use of corticosteroids etc. In addition to complete ophthalmic examination, diagnostic modalities like culture and sensitivity test and cytology were suggested (Wilkie and Whittaker, 1997).

Whitley (2000) divided the deep stromal ulcers into progressive and non-progressive types. Progressive deep stromal ulcers in the dog were potentially a threat to vision and a detailed investigation should be carried out.

Deep ulcerations extend to one-half the stromal depth or greater, required corneal vascularisation for healing. The healing time was about three weeks (Moore, 2003).

Ollivier (2003) opined that the nonprogressive stromal ulcers could be managed medically in the same manner as superficial ulcers, with the possible addition of a surgical treatment like conjunctival graft or flap. These complicated deep ulcers may lead to impaired vision because of cornea1 scaring or anterior synechia, or lead to loss of the eye because of endophthalmitis, glaucoma, phthisis bulbi, or the combination of these.

According to Mandell and Holt (2005), deep stromal ulcers required vascularization for healing and may take three weeks to heal.

2.4.2.4. Melting ulcer

Whitley (2000) described melting ulcers as Collagenase- and Protease-associated ulcers. Proteases and collagenase aid in the removal of devitalized cells and debries from the cornea in the corneal healing. Corneal epithelial cells, fibroblasts, polymorphonuclear leukocytes, some bacteria (*Pseudomonas* spp.) and some fungi produce protease and collagenase. These enzymes contribute to the progressive breakdown and rapid melting of the corneal stroma in some corneal ulcers.

Ollivier (2003) opined that melting ulcers with progressive stromal dissolution were not a specific disease; rather, they are a complicating component of corneal ulcers. Ulcers with highly active proteases have a greyish, gelatinous appearance, which must be distinguished from corneal oedema.

Melting ulcers were characterized by progressive stromal dissolution secondary to proteolytic activity (Vanor *et al*., 2007).

Gilger et al. (2008) described melting ulcer as a complicating component of corneal ulcer. The damaged corneal cells as well as microorganisms produce protease and collagenase to aid in removal of devitalized cells and debris from the cornea, but if in excess these protineases contribute to the progressive break down and rapid melting of corneal stroma. Protease inhibitors may reduce the progression of stromal ulcer, speedup epithelial healing and minimize corneal scarring, and include autologous serum, N-acetyl cystein, EDTA, tetracycline etc.

2.4.2.5. Descemetocele

Startup (1984) opined that descemetoceles were very deep ulcers with exposed Descemet's membrane and impending perforation of the globe. It could

be recognized by clear appearance of the membrane and its lack of fluorescein dye retention.

Wilkie and Whittaker (1997) opined descemetocele as a complication of corneal ulcer.

Whitely (2000) stated descemetocele as a very deep ulcer with exposed Descemet's membrane, which is an ocular emergency and requires immediate surgical intervention.

Descemet's membrane is always under some tension and may herniate through the surrounding corneal stroma to bulge anteriorly to form descemetocele. Fluorescein retention occurs in the surrounding exposed stroma only. Descemet's membrane often appears clear or black (Mandell and Holt, 2005).

2.4.2.6. Corneal perforation

Wilkie and Whittaker (1997) reported that corneal perforation occurred as a result of a complicated corneal ulcer.

Sansom (2000) opined that penetration of anterior chamber causes loss of aqueous humor and acute pain and later the fibrin clot seal the wound. In iris prolapse, a distorted pupil in the direction of corneal wound could be seen and such wounds resulted in shallowing of anterior chamber, uveitis, miosis, aqueous flare and hyphaema.

Miller (2001) stated that Descemet's membrane is a significant barrier to perforation from deep corneal ulcers and resistant to enzymes that denature overlying stroma. After corneal perforation, enophthalmitis is the serious threat. Immediately after perforation, iris flows forward into the wound to seal the hole.

Mandell and Holt (2005) opined corneal perforation as a surgical emergency with the clinical signs like blepharospasm, pain, corneal oedema, a

misshapen cornea, and a pink or red tissue. There may be change in the depth of anterior chamber and dyscoria. The iris may adhere to the rent in the cornea or may prolapse. Restraint or any pressure on the neck or jugular vein might cause increased intraocular pressure and corneal rupture. Seidel test can perform to check for leakage of aqueous humor. Corneal perforation could be treated with broadspectrum antibiotics, NSAIDS, mydriatics and cycloplegics.

According to Gilger *et al.* (2008) conjunctival tissue may not have adequate strength to maintain a water tight seal and a formed anterior chamber after surgery in a full thickness corneal defect. They advised to use cornea or another material having more structural integrity than conjunctival tissue, to overcome these problems.

2.4.3. Etiology

According to Startup (1984) any break in the continuity of the intact corneal epithelium allowing the entry of microorganism may result in ulceration like corneal trauma from scratch, accidents, entropion, distichiasis, conjunctival lithiasis, keratoconjunctivitis sicca, foreign body in cornea or third eyelid, tumour in eyelid margin, corneal drying etc.

Bedford (1987) opined that untreated proptosis might lead to exposure keratitis and corneal ulcers.

Melting corneal ulcers in the dog may result from the action of proteolytic substances on the corneal stroma. These ulcers, often of traumatic origin, then infected by opportunistic bacteria, could melt in to very rapidly (Wolfer and Grahn, 1994).

Massa *et al.* (1999) stated that numerous types of gram positive and gram-negative organisms were identified as causative agents of infectious ulcerative keratitis in people, dogs and horses.

Moore (2003) opined that disorders that interfere with epithelization, basement membrane formation, or adherence between epithelial cells, basement membrane and stroma were the reasons for indolent corneal ulcers. Lagophthalmos, abnormal eyelid conformation or cranial nerve deficit could also lead to exposure keratitis and secondary chronic corneal epithelial defect.

Ollivier (2003) described that the corneal ulcers were frequently traumatic in origin and were not primarily infected, but rapidly contaminated by bacteria.

Mandell and Holt (2005) described the causes of corneal ulcer, although usually of traumatic in origin, eyelid conformation or an underlying disease process may also predispose an animal to corneal ulcer. They enlisted the causes as trauma, foreign body, infection, Keratoconjunctivitis Sicca, topical irritants, exposure keratitis and entropion.

Ledbetter *et al*. (2006) stated the etiologies of refractory corneal ulcers as morphologic and neurologic abnormalities of the eyelids, aberrant eyelashes or facial hair, quantitative or qualitative tear film abnormalities, deficiencies of corneal innervations, foreign bodies, and microbial infection.

2.4.4. Clinical signs

Pain, Lacrimation, epiphora, blepharospasm, photophobia of varying degrees, and oedema were the predominant signs in ulcerative disorder (Renwick, 1996).

The clinical signs of corneal diseases were epiphora, blepharospams, photophobia, pawing and opacification (Wilkie and Whittaker, 1997).

Murphy *et al*. (2001) noted Spontaneous Chronic Corneal Epithelial Defects characterized by the presence of epithelial erosion surrounded by a circumferential sheet of loosely adherent or nonadherent epithelial cells (“epithelial lip”). The affected dog displayed variable discomfort evidenced by

blepharospasm and epiphora. The defects were chronic, with some persisting for more than 6 months.

Morreale (2003) reported the primary sign of corneal diseases, as loss of clarity, pigmentation, fibrosis, accumulation of cellular or non cellular infiltrate and oedema.

According to Ollivier (2003) the ocular signs of ulcerative keratitis included pain, epiphora, purulent discharge, photophobia, hyperemia of conjunctiva, and corneal changes and associated uveitis.

Clinical signs of corneal ulceration included blepharospasm, conjunctival hyperemia, a relatively clear ocular discharge, corneal oedema, and miosis. A yellow–white stromal infiltrate usually accompanies infected ulcers (Mandell and Holt, 2005).

Janssens (2007) described the clinical signs of indolent ulcers as photophobia, blepharospasm, epiphora and moderate pain, which decreases with the chronic nature of the erosion.

2.4.4.1. Corneal opacity

According to Bedford (1982) corneal opacity was related to inflammation, oedema, dystrophy or scar formation and it could be identified with ease using focal illumination.

Corneal fluids were hypotonic to both tears and aqueous humor. Any break in either epithelium or endothelium, whether focal or diffuse, increased the fluid content of that cell and lead to corneal oedema (Startup, 1984).

Wilcock (1993) opined that the oedema occurs rapidly following injury and resulted from inhibition of lacrimal water flow through the damaged corneal epithelium or failure of extrusion of electrolytes by the corneal endothelium and such oedematous cornea appeared clinically opaque.

Wilkie and Whittaker (1997) reported that the disruption of the regular lamellar arrangement of stroma or changes in the collagen type appeared as opacity. Dehydrated state of cornea was maintained by corneal epithelium and endothelium. Break in these barriers resulted in corneal oedema.

According to Bentley (2005) any corneal oedema will be confined to the area of the erosion. Diffuse stromal oedema implied endothelial disease, with secondary corneal oedema and bullous keratopathy, which was more likely underlying cause of the erosion.

Corneal oedema resulted from imbibitions of fluid by the epithelium or stroma. Corneal oedema regarded as the increased water content that resulted in increased thickness, and scattering of light, and reduced transparency (Gilger *et al*., 2008).

2.4.4.2. Neovascularisation of cornea

Magrane (1977) observed vascularization of cornea was in response to various pathologic processes and in stromal healing.

Renwick (1996) reported the vascularisation as superficial, where blood vessels were seen crossing the limbus and branching over the surface of the cornea and deep, where the vessels were restricted by the corneal lamellae and hence tend to be straight rather than arborising. They cannot be seen crossing the limbus and often gives a 'brush border' effect.

The incidence of deep corneal vascularisation was depending upon the duration of ulcer. The vessels usually developed after three to five days and progressed less than one millimetre per day (Wilkie and Whittaker, 1997).

According to Moore (2001), corneal blood vessel pattern vary with the type of keratitis present. Long branching vessels were consistent with a superficial ulcerative or nonulcerative keratitis. Deep corneal neovascularisation

appeared as fine non-branching vessels and was associated with deep keratitis. Deep corneal vessels forming a 360^{o} perilimbal pattern were seen with intraocular disease.

Murphy *et al*. (2001) described the correlation of an increased vascular response with increased proximity to the limbus suggested the elaboration of vasculogenic factors from peripheral wound beds were more likely to reach the perilimbal vasculature at a critical concentration that stimulates neovascularization.

Although corneal neovascularisation was beneficial in the early healing stages of ulcerative keratitis, an excessive degree could cause ocular discomfort and corneal opacity. Vascularization, together with graft size (more than 8 mm) and proximity to the limbus were important factors adversely affecting graft survival in penetrating keratoplasty (Featherstone *et al*., 2001).

According to Bentley (2005) central corneal lesions commonly existed weeks to months without any vascular response where as peripheral lesions resulted in more vascularisation of cornea.

2.4.4.3. Corneal pigmentation

Magrane (1977) observed that uveal pigment migrated through nerve and vessel opening and deposited in the corneal stroma, following the development of anterior synechiae in corneal perforation and iris prolapse. Also opined that uveal pigment should not be removed surgically since it disrupted synechiae and produced aqueous humor leakage.

Renwick (1996) described pigment deposition as a sign of chronicity. Pigmentary keratitis was most commonly encountered in brachycephalic dogs. Corneal pigment deposition and any associated vascularisation generally commenced at the medioventral quadrant and advanced axially. There was often

little or no sign of irritation and the degree of pigmentation become so great as to cause blindness.

Slatter and Dietrich (2003) reported that epithelial pigmentation was more common in chronic corneal diseases due to exposure, or nasal fold trichiasis and distichiasis. Stromal pigmentation was associated with more severe corneal diseases and vascularisation. In chronic exposure, corneal epithelium might get reverted to skin pattern with keratinisation and pigmentation.

In dogs, melanin pigmentation of the anterior stroma might have occured with an ulcerative keratitis. This pigmented migration was associated with a chronic inflammatory response and progressed from the peripheral cornea to the centre. The most common clinical signs accompanying corneal sequestration included epiphora, blepharospasm, and photophobia. Corneal vascularization and interstitial keratitis were also observed (Bouhanna *et al*., 2008).

According to Gilger *et al*. (2008) corneal pigmentation was usually associated with chronic inflammation and irritation. Corneal pigmentation resulted from migration of melanocytic cells from the limbal and perilimbal tissues which were deposited in the basal epithelial cells and anterior stromal tissue and usually accompanied with corneal vascularization, stromal inflammatory cell infiltration, and granulation tissue formation.

2.4.5. Ophthalmic examination

Bedford (1982) opined that oblique illumination was effective in determining corneal epithelial loss and posterior synechiae.

Direct diffuse illumination could assess corneal contour, clarity, symmetry and contact between the eyelid margins and cornea, while direct focal illumination was useful in determining the location and extend of corneal lesions (Miller and Crenshaw, 1988).

Wilkie and Whittaker (1997) opined that the culture and sensitivity, Schirmer's tear test, cytology, fluorescein stain retention, and complete anterior segment examination should be taken as routine examination in animals with corneal ulcer.

Massa *et al*. (1999) opined that the diagnostic specimens obtained from animals with ulcerative keratits typically included swab specimens for aerobic microbial culture and scrapings for cytologic evaluation.

Cornea should be examined with a good light source such as penlight. The location, colour, shape and pattern of corneal lesions were helpful in providing information about the underlying cause (Moore, 2001).

Ophthalmic examination usually preceded general examination and closer eye examination should commence in a dimly lit room, in order to obtain information about pupil size of each eye and the transparency of individual ocular layers. The ocular adnexa, conjunctiva and cornea should be examined with direct and oblique illumination using focusing flashlight or the direct light beam of the ophthalmoscope. (Beranek and Vit, 2007)

2.4.6. Ocular Reflexes

According to Magrane (1977) the pupillary light reflex was helpful in determining optic nerve and retinal function. The direct and indirect reflexes were recorded as normal, sluggish, incomplete, or absent.

Felchle and Urbanz (2001) reported that pupillary light reflex test was effective in evaluating the function of retina, optic nerve and the iris sphincter muscle.

Menace response, a threatening, sudden movement presented near the eye, which elicited a blink. Before testing for menace test, be sure that the facial

nerve was intact by eliciting a blink reflex through the palpebral or corneal reflex (Martin, 2001).

A common clinical test to assess vision was the menace reaction conducted by passing the hand in front of the animal's eyes to induce blink reflex and animal's response was evaluated. Occasionally, dogs and cats did not show any response with these tests. The response of the pupil was also used as a sign of visual competence (Beranek and Vit, 2007).

Ollivier *et al*. (2008) stated that menace test evaluates the function of optic nerve, the facial and abducent nerve. This test was performed by making a menacing gesture with hand towards eye, without touching the vibriassae or causing excessive air currents, which may results in false positive result. If the animal is able to see, it should blink or move its head away.

2.4.7. Schirmer's Tear Test.

Bedford (1982) opined that fifteen millimetres of a standard filter paper strip should become wet over a period of one minute, when placed in lower conjunctival sac of a normal dog.

Miller (2001) stated that Schirmer's tear test was indicated in corneal ulcer because a normal tear film was required to maintain an intact corneal epithelium.

Munro (2001) reported that Schirmer's tear test should be performed in unsedated patients early on ophthalmic examination and prior to any topical drug administration. Sedation, general anaesthesia, topical anaesthesia and parasympatholytic agents will significantly reduce the reading and most normal dogs have reading of 14 mm/minute or greater.

Murphy *et al.* (2001) described the method of estimating tear production by placing Schirmer's tear test strip in the conjunctival sac in dogs.

Morreale (2003) described Schirmer tear test (STT) as the most common test for pre corneal tear film and it asses the quantitative production of the aqueous portion of the tear film, which commonly performed before topical anaesthesia. Also stated that the normal value of Schirmer's tear test in a dog ranged from 14 to 25 millimetres per minute, a Schirmer tear test result of less than 15 millimetres per minute is under suspicion, and less than 10 millimetres per minute is indicative of keratoconjunctivitis sicca.

Beranek and Vit (2007) described Schirmer tear test that measured production of the aqueous portion of the tear film. The sterile strips would not negatively influence subsequent bacteriological or fungal examinations.

2.4.8. Fluorescein Staining

Bedford (1982) described the use of fluorescein can to identify the corneal ulcer or erosion. The dye cannot penetrate the epithelium, but readily taken up by the stromal tissues and the area will be stained a brilliant green.

Miller and Crenshaw (1988) advocated the use of fluorescein dye strips for determining corneal integrity.

According to Wilkie and Whittaker (1997) the hydrophilic nature of corneal stroma was responsible for the retention of the water soluble sodium fluorescein dye.

The water soluble fluorescein stain does not penetrate the intact hydrophobic corneal epithelium but in epithelial defect, the hydrophilic corneal stroma would be stained green (Moore, 2001).

Miller (2001) described fluorescein stain as the primary test for diagnosing corneal ulcers, and the most commonly used form of fluorescein stain was the impregnated paper strip. The strip was moistened with physiological

saline or eyewash and touched to the superior bulbar conjunctiva and allowed the animal to blink several times to distribute the stain across the cornea, and excess stain was gently flushed from the eye. Fluorescein stained intracellular spaces in the corneal stroma gaining access through and outlining breaks in the epithelium.

When there was a break in epithelium, the hydrophilic sodium fluorescein dye with the property of fluorescence, would be repelled by the lipophilic corneal epithelium and absorbed by water soluble intracellular spaces. Fluorescein did not stain the descemet's membrane even if it was exposed but stained the wall (Morreale, 2003).

Ollivier (2003) described the use of fluorescein stain for the identification of corneal ulceration or to evaluate the corneal integrity.

Fluorescein staining, one of the most common staining techniques, and indicated when there was evidence of corneal injury or other discontinuity of the corneal surface or any pain of unknown origin. Corneal defects appeared green, particularly when cobalt filter or Wood's lamp (ultraviolet light) were used. The staining of the eye was transient and usually disappeared within 45 minutes time (Beranek and Vit, 2007).

2.4.9. Culture and sensitivity test

Bedford (1982) opined ocular discharge should always be submitted for microbiological examination and related antibiotic sensitivities.

Miller and Crenshaw (1988) opined that the specimens for microbial culture should be obtained before adding a topical anaesthetic, because ophthalmic topical contain preservatives and antimicrobial agents, which will inhibit microbial growth in culture.

Wilkie and Whittaker (1997) opined that culture and sensitivity should be considered for all corneal ulcers and perforations.

Massa *et al.* (1999) used sterile swabs to collect material from the centre and periphery of the epithelial defect and found out that *Streptococcus* spp. and *Staphylococcus* spp. as the most prevalent bacterial organisms,followed by *Pasteurella* spp. and *Pseudomonas* spp.. The most common fungal isolates belonged to *Aspergillus* spp. and *Candidan* spp. Also opined that cultural studies helped in making definitive diagnosis and also for designing the treatment regimen. Topical use of antimicrobials interfered with the ability of the test to obtain definite diagnosis.

Whitley (2000) observed that the deeper stromal ulcers were commonly infected and melting corneal ulcers were always infected. The culture studies involving corneal ulcers in dogs isolated *Staphylococcal* spp. (39%), *streptococcus* spp. (25%), *Pseudomonas* spp. (9.4%), *E. coli* (4.7%), *Corynebacterium* spp. (3.9%) and *Bacillus aureus* (2.4%). Chloramphenicol, gentamicin, amikacin and ciprofloxacin were found effective in most of the infections.

Morreale (2003) opined that corneal culture and sensitivity test should be used for the selection of antibiotic therapy. Corneal infections in dogs and cats were most commonly caused by aerobic bacteria, especially isolates of *Pseudomonas* spp. and *Staphylococcus* spp.

According to Ollivier (2003) culture and sensitivity testing provided useful information for the diagnosis and determination of appropriate antimicrobial therapy in cornea1 diseases. Cultures should be obtained from any deep, melting, and progressive or otherwise infected-appearing corneal ulcer.

Varges *et al*. (2009) studied the antimicrobial susceptibility of *Staphylococci* isolated from naturally occurring canine external ocular diseases and highlights the presence of *Staphylococcus* spp. in naturally occurring extra-ocular canine ocular disease and the emergence of resistant strains to common antimicrobial drugs. It also emphasized the need for bacterial culture with species

identification and susceptibility testing in order to choose the appropriate antimicrobial therapy. In their study enrofloxacin was the most effective fluoroquinolone (25% resistance), followed by ciprofloxacin (30% resistance).

2.4.10. Microflora in cornea.

Bedford (1987) stated that the exposed corneal stroma was very much susceptible to secondary infection, with *Staphylococci*, *Streptococci*, *E. coli* and *Pseudomonas* being the commonest organisms encountered.

Hamor (2001) suggested the basic thumb rule that the gram-positive species were more likely normal flora and gram-negative species were more likely pathogenic. However, the heavy growth of a gram-positive species was likely significant and should be treated.

Ollivier (2003) described that Gram-positive organisms were most commonly isolated in the normal flora of the canine and feline eye. *Staphylococcus* spp., *Streptococcus* spp., *Corynebacterium* spp*., Bacillus* spp., *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella, Neissaria* spp., and *Fusobacterium* spp. were the only anaerobic bacteria that occasionally found in the normal canine eye.

Mandell and Holt (2005) reported that gram positive and gram-negative organisms were capable of infecting the cornea. Gram-negative rods produce proteases (collagenases), which could result in rapid progressive destruction ("melting") of corneal tissue.

Wang *et al*. (2008) described that *Staphylococcus* spp. were the most frequently isolated microorganisms from dogs with corneal ulceration. *Staphylococcus intermedius* was the most common isolate in the conjunctival sac of clinically normal dogs and dogs with ulcerative keratitis in China, so *Staphylococcus intermedius* belonged to resident flora in the conjunctival sac of normal dogs. If corneal ulcer developed , regardless of causes, *Staphylococcus*

spp., *Streptococcus* spp. and *Pseudomonas* spp. are likely to invade the ocular surface of dogs in Beijing, China.

Ledbetter and Scarlett (2008) reported that all corneal lesions from which anaerobic bacteria isolated were stromal ulcers of different depths. Under normal physiologic conditions, the cornea would not expect to support the growth of obligate anaerobic bacteria; however, a corneal microenvironment with low oxygen concentration permissible to the survival of these bacteria may develop under a variety of circumstances.

2.4.11. Haematology

According to Schalm *et al.*(2000) normal values of various haematological parameters such as total leukocyte count, neutrophils 60-77 per cent (av 70%), band cells 0-3 per cent (av 0.8%), lymphocytes 12-30 per cent (av 20%), monocytes 3-10 per cent (av 5.2%), eosinophils 2- 10 per cent (av 4.0%) and basophils rare. Haemoglobin concentration will be between 12 and 18 g/dl (av 14.9g/dl) and packed cell volume 37 -55 per cent (av 45.54%).

Raji (2006) reported that the hematological parameters did not vary much from the normal range in dogs with corneal affections.

The hematology in animals with keratitis did not vary much from the normal range to demonstrate any underlying disease process (Resmi, 2008).

2.4.12. Treatment

Treatments of corneal ulcers depend upon their depth and rate of progression. The general therapeutic principles apply to treat the ulcer, superficial or deep, but rapidly progressing deep stromal ulcers warrant surgical intervention in order to give support and reduce the risk of perforation (Renwick, 1996).

According to Ollivier (2003) the therapeutic strategy in bacterial keratitis was to eradicate the infection, reduce or stop the cornea1 destruction and support the cornea1 structures, control of uveal reaction and the pain associated with it, and minimize the scarring.

2.4.12.1. Medical treatment

Startup (1984) described the usefulness of broad-spectrum antibiotics and atropine as a cycloplegic agent. Also advocated to start anticollagenases such as acetyl cysteine, wetting agents such as methylcellulose based preparation in cases of tear film deficiencies as the treatment of corneal ulcer. Treatment with vitamin A, orally or local application is beneficial especially in corneal erosion syndrome and vitamin C and E proved effective in cases of indolent ulcers.

Roberts *et al.* (1986) studied the disinfecting property of povidone-iodine concentrations of 1:2, 1:20, 1:10, 1:50, 1:100 in eye. It was concluded that 1:50 povidone- iodine solution was effective in eliminating bacterial contamination of the external ocular tissue without tissue reaction

According to Bedford (1987) topical and systemic broad spectrum antibiotics together with cycloplegics and mydriatics therapy were essential for treatment of corneal perforation.

Miller (1996) used polysulphated glycosaminoglycans (PSGAG) to treat indolent ulcers in dogs. 5% topical solution was effective in treating 83% of the cases studied. Polysulphated glycosaminoglycans had been demonstrated to concentrate in ocular tissue after intramuscular injection.

According to Collins *et al*. (1995) high intervention of cornea caused substantial postoperative pain, analgesics were essential to improve animal′s comfort and to minimize the tendency to self trauma. The potential benefit of anticholinergic treatment was prevention or reversal of oculocardiac reflex. In ketamine administration the palpebrae remain opened, globe was centrally

positioned and the palpebral and corneal reflex persisted. Diazepam reduced the ketamine induced increase in intraocular pressure. Topically administered anaesthetic may be irritating and can cause transient conjunctival hyperaemia.

According to Wilkie and Whittaker (1997) the medical treatment for corneal ulceration included topical application of artificial tears, broad spectrum antibiotics, mydriatics-cycloplegic agents and anti-inflammatory or immune modulating drugs.

Munro (2001) described that the antibiotics were indicated in the treatment of corneal ulceration, as the loss of the epithelial barrier exposed the corneal stroma. The topical application provided therapeutic concentration of the drug and markedly increased penetration in corneal epithelial cells. Flurbiprofen had good corneal penetrability and used to control uveitis after intraocular procedures.

Giuliano (2004) observed that topical NSAIDS represented a more selective modality to treat ocular inflammation and a more efficient therapeutic choice for treating ulcerokeratouveitis or stromal abscesses and controled postoperative pain and inflammation after intraocular procedures.

Effective management of ocular bacterial infections required identification of microorganisms, and good knowledge of the mechanism of action and therapeutic characteristics of antibiotics. Fluroquinolones were found effective against *Staphylococcus* spp. and *Pseudomonas* spp. (Kern, 2004).

Willis (2004) reviewed various drugs used in glaucoma in small animals. Two percent dorzolamide significantly reduced intraocular pressure and aqueous humor flow in dogs.

Mandell and Holt (2005) opined that the emergency treatment of corneal ulcers should be based on the depth of the ulcers. The medical therapy for

corneal ulceration should be focused on the relief of pain and infection. The topical medication should include 1% atropine, antibiotics and NSAIDS.

Ledbetter *et al.* (2006) found that the topical therapy with an antimicrobial - Chondroitin sulphate solution combined with manual debridement was effective. But Tobramicin-Chondroitin sulphate Solution and Ciprofloxacin-Chondroitin sulphate ophthalmic solutions appeared to be ineffective in the treatment of ulcerative keratitis associated with bullous keratopathy

Townsend *et al.* (2009) reported that topical fluroquinolones were effective in treating infected corneal ulcers or stromal abscesses especially against gram negative organisms.

Hendrix and Cox (2008) studied the pharmacokinetics of topical ciprofloxacin in tears and concluded that mean tear concentration of ciprofloxacin remained above the MIC_{90} levels for most pathogenic bacteria for 6 hours in normal mesocephalic and brachycephalic dogs.

2.4.12.2 Surgical treatment

According to Bedford (1980), membrana flapping in which the membrana nictitans will be sutured to the dorsal bulbar conjunctiva as a simple and effective way of covering and protecting the whole corneal surface. Fornix based conjunctival hood flaps and the pedicle conjunctival flap or grafts were used to cover specific areas of the cornea.

Helper (1981) described the procedure for temporary tarsorrhaphy. One or two interrupted horizontal mattress sutures were used for it and a small opening will be left at the medial canthus to facilitate medication.

Startup (1984) described various techniques for the treatment of corneal ulcers, which included conjunctival flaps, third eyelid flaps, hydrophilic contact lenses, and superficial keratectomy.

Pickett (1995) opined that superficial keratectomy where the epithelium, the abnormal basement membrane, and a part of the underlying stroma were removed to allow the migration of normal epithelium and deposition of normal basement membrane was a better option in persistent corneal erosions.

Wilkie and Whittaker (1997) described the complications of superficial keratectomy, as delayed healing, infection, recurrence of the lesion, granulation, pigmentation, perforation and scar formation. These complications were mainly observed in brachycephalic breeds.

Stanley *et al*. (1998) reported that the complications of superficial keratectomy included perforation, permanent scar formation, infection, corneal vascularisation and fibrosis.

Hansen and Guandalini (1999) stated that the principle advantage of the techniques lies in the ready availability of material to fill and strengthen the stromal defect. The techniques available to treat the corneal lesions included cyanoacrylate tissue adhesive, conjunctival pedicle graft, conjunctival island patch, tarsoconjunctival graft, lamellar corneal grafts, and penetrating keratoplasty. They studied and concluded that frozen lamellar corneal graft was a very safe technique for deep perforating ulcers of cornea.

Refinements of microsurgical techniques including the use of cyanoacrylate glue and porcine small intestinal submucosal grafts had been advocated for the surgical treatment of deep comeal ulcers (Munro, 2001).

Deep corneal defects should be managed surgically for optimum results and techniques available included conjunctival autografts, cyanoacrylate tissue adhesive, corneal–scleral–conjunctival transpositions, lamellar keratoplasty, and penetrating keratoplasty. The tectonic corneal grafts and the non-corneal grafts including split-thickness dermal grafts, equine pericardium, peritoneum, equine amniotic membrane, human amniotic membrane in rabbits, equine renal capsule, and expanded polytetrafluoroethylene were used (Featherstone *et al*., 2001).

Hollingsworth (2003) suggested the excision of severely contaminated or extensively damaged prolapsed iris in staphyloma. To provide support to deep corneal ulcers or keratectomy sites and there by preventing corneal rupture, the conjunctival grafts were suggested. While suturing the corneal wounds corneal sutures should not pass through the entire thickness of cornea.

Morgan (2004) described steps for preparation of eyelid surgeries. The eyelids and adnexa were gently wiped with gauze soaked in diluted aqueous povidone-iodine solution (diluted to 1:10 to 1:50 with 0.9 % irrigating saline.) alternated with gauze soaked in saline solution. Sterile cotton swabs soaked in diluted povidone-iodine solution might be used to remove residual material from conjunctiva and ocular surface.

Ollivier *et al*. (2006) described that because of the risk of corneal melting and infection by opportunist pathogens after keratectomy and irradiation, it had been recommended to cover the corneal defect created by keratectomy, which included permanent bulbar conjunctival grafts, corneal grafts, porcine small intestinal submucosa (SIS) grafts, and amniotic membrane (AM) transplantation.

Bouhanna *et al*. (2008) opined medical treatment was generally unsatisfactory for stromal sequestration and recommended conjunctival flap and corneoconjunctival transposition, especially with deep ones. Tsuzuki *et al.* (2008) suggested the success of glycerin-preserved porcine amniotic membrane transplantation onto the canine cornea was due to the result of early collagen matrix reorganization. This technique therefore appeared to be a potential alternative in the treatment of ocular surface disorders.

2.5.STAPHYLOMA

The extrusion of iris sealed the corneal wound but provided a potent tract to intraocular infection. Such wounds should be repaired as early as possible so that the risk of staphyloma formation and risk of infection could be reduced.The use of membrane flap for corneal wounds was also advocated (Bedford, 1987).

The iris protruded as a brown or black mass through the corneal perforation, thus resulted in iris prolapse/staphyloma. The clinical signs of iris prolapse were blepharospasm, corneal oedema, a misshapen cornea, a fibrin clot, change in anterior chamber depth and dyscoria (Mandell and Holt, 2005).

Townsend *et al*. (2009) described that staphyloma was the protrusion of uveal tissue through thinned cornea or sclera. It initially appeared black or slightly brown due to a covering of fibrin, subsequent granulation and scar formation made the colour to a vascular gray.

2.6. COLLAGEN IN CORNEAL HEALING

Geggel *et al.* (1985) described the criteria for an ideal ocular surface substrate as, easiness of surgical manipulations; induce minimal inflammation; and be nontoxic to epithelial cells. Collagen had been employed in ophthalmology for a variety of uses and the eye tolerates various collagen preparations quite well. Corneal epithelial cells, as well as other epithelial cells, growed better on collagen than on uncoated tissue culture plates.

According to Helper (1989) cornea consisted of large amount of collagen, one of the factors involved in corneal wound healing.

Shaker *et al*. (1989) studied the effect of collagen bandage lenses on corneal epithelial wound healing after mechanical debridement of the cat cornea. Shield dissolution appeared to involve both enzymatic and mechanical action and the effect was found to be most pronounced during the first eight hours after debridement. The rate of shield dissolution was thought to be depending on several host factors, including tear volume, tear enzyme concentration, degree of inflammation and blink rate. One major advantage over the hydrophilic lenses was higher oxygen permeablility through collagen which also increases as collagen biodegraded.

Gelatt (1991) described the use of collagen inserts for drug delivery. It was opined that when collagen was exposed to aqueous media, hydrolytic changes took place leading to the release of the drug. Also mentioned about the use of collagen shield of porcine origin, which was useful in treating various ocular surface disorders. It was reported that its effect was more pronounced during the first eight hours of therapy.

Geasey *et al*. (1992) reported that collagen shields were proved to be highly oxygen permeable, promote epithelial healing, decrease inflammatory cell infiltration and reduced stromal oedema. He opined that dissolution of the shield occurred by tear enzymes, mechanical action of the eyelid and by the cellular phagocytic activity.

Gelatt and Gelatt (1994) stated that irrespective of the nature of the graft structure, the structure of the recipient bed had to be simulated in the graft as it acts as a scaffold for the host corneal cells to migrate, as a source of corneal stroma and collagen lamellae and not as a source of viable cells.

Barros *et al*. (1998) reported that the xenologous amniotic membrane served as a substrate for the reestablishment of the corneal layers. Also stated that the amniotic membrane was effective to restore the globe integrity, stabilized the inner contents, preserved vision, and to prevented endophthalmitis in corneal perforations.

Barros *et al.* (1999) reported corneal repair using synthetic or biological materials and studied the use of preserved equine pericardium in the repair of superficial defects of the cornea of the dog. The epithelialization began on seventh day and was completed at day 30. The opacification at the graft site could be minimized when treated by third eyelid flap, contact lens and temporary tarsorrhaphy.

Lewin (1999) described the porcine small intestinal submucosa (SIS) as acellular and composed mainly of extracellular connective tissue. It was reported the use of SIS as a vascular graft, for the repair of bladder wall and Achilles tendon etc., where regeneration of the recipient organ resulted. In no case immune rejection of the xenograft was observed since it was acellular collagen which was same between the species.

Featherstone and Sansom (2000) described small intestinal submucosa (SIS) as a biodegradable, collagen-based material derived from the submucosal layer of porcine small intestine. SIS is acellular, non-immunogenic, xenogeneic and had demonstrated good regenerative capacities in multiple organ systems.

According to Featherstone *et al*. (2001) the ideal biomaterial for the repair of corneal defects should achieve strict specifications for optical clarity, support of epithelial migration and adhesion, permeability to solutes, and stability to corneal proteases. Potential biomaterials included collagen, collagen-hydrogel copolymers, bioactive synthetics, and coated hydrogels.

Bussieres *et al*. (2004) used the porcine small intestinal submucosa for the repair of full thickness corneal defects in dogs, cats and horses, showed that collagen based material had the advantage of being cost effective, commercially available and easy to handle. They reported the use of autologous, homologous, and xenologous biomaterial grafts for repair of lamellar keratoplasty.

Biological membranes had been used as an alternative surgical therapy to repair defects of the ocular surface for several corneal and scleral conditions. Amniotic membrane was used to repair full thickness and lamellar lesions with good results. It reinforced adhesion of basal epithelial cells, promoted epithelial differentiation, and reduced epithelial cell apoptosis, diminished antiprotease activity, and minimized corneal scarring (Barros *et al.*, 2005).

Ollivier *et al*. (2006) described the placement of an amniotic membrane material represented an alternative surgical procedure to bulbar conjunctival

grafts, especially if there was a lack of bulbar conjunctiva tissue available after tumor resection or if a particularly large corneal resection was necessary. The amniotic membrane incorporated into the corneal defect seemed to create much less scarring than a corneal defect covered by bulbar conjunctiva.

According to Raji (2006) the collagen diskettes prepared from bovine Achilles tendon were effective in promoting healing of corneal ulcers. However, difficulty was encountered in application and retention of the collagen diskettes and the edges were cut in 'v' shape and adjusted to avoid the space inside.

Vanor *et al*. (2007) described the three important stages in SIS integration and corneal wound healing: as corneal neovascularization, proliferation of epithelial and stromal tissue, and remodeling of the extracellular matrix (ECM) to produce corneal transparency and preserved corneal integrity.

Tsuzuki (2008) described the reconstructive transplantation with porcine amniotic membrane as a potential alternative in the treatment of ocular surface disorders and the success was due to the early collagen matrix reorganization.

Kim *et al.* (2009) reported that Amniotic membrane possessed several beneficial features that encouraged epithelization and inhibit fibrosis during the treatment of corneal disease. They demonstrated that bovine freeze dried-amniotic membrane would be an effective alternative treatment for canine superficial corneal ulceration and suggested that such a treatment will be better than other conventional therapies for the condition.

Plummer (2009) reviewed amniotic membrane transplantation for ocular surface reconstruction in 58 equine cases and suggested amniotic membrane transplantation as a treatment option in cases that required ocular surface reconstruction for the treatment of corneal and conjunctival defects either organic or surgically induced.

Materials and Methods

3. MATERIALS AND METHODS

The study was conducted in twelve dogs of different age, breed and sex presented to Veterinary College Hospitals at Mannuthy and Kokkalai.

3.1. SELECTION OF CASES

All the dogs presented with ophthalmological complaints were thoroughly examined and twelve dogs with corneal ulceration / laceration / penetrating wound / staphyloma were selected for the study. They were divided into Group I and Group II each consisted of six animals (Plate 1 and 2).

In Group I consisting Dog Nos. I, II, III, IV, V and VI, the corneal lesions were protected with collagen sheet of bovine origin after initial treatments like debridement / scarification / keratectomy. In Group II consisting Dog Nos. VII, VIII, IX, X, XI and XII, the corneal lesions were treated by debridement / scarification / superficial keratectomy.

The post operative clinical observations were made at weekly intervals for four weeks and later fortnight intervals up to 60^{th} day to study the effectiveness of the treatment.

3.1.1. Signalment and Anamnesis

The age, sex, breed, symptoms noticed by the owner, duration of illness and details of previous medications were recorded.

3.1.2. Preoperative therapy

The preoperative therapy consisted of ocular instillation of antibiotics (ciprofloxacin[1]) and anti-inflammatory agents (flurbiprofen[2]).

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1. Ciplox eye drops (0.3%), Cipla Ltd, Verna, Goa
2. Flur (0.03%), Nicholas Piramal, Dhar, Madhya Pradesh

The antibiotic selected initially was ciprofloxacin and was changed, when required, according to the results of culture and sensitivity test of corneal swab. Eye drops were instilled four times daily with an interval of 10 minutes between the medicines. In all cases oral cephalexin[3] at the rate of 20 mg/kg body weight twice daily for five days and multivitamins were advised.

The oral administration of carbonic anhydrase enzyme inhibitors (acetazolamide[4]) at the rate of 20 mg/kg body weight and ocular instillation of dorzolamide[5] eye drops or of beta blockers (timolol maleate[6]) were advised in cases where intraocular pressure on the opposite eye was found elevated.

3.2. SURGICAL TREATMENT

3.2.1. Preoperative Preparation

All the dogs were put under medical therapy with topical application of ciprofloxacin eye drops prior to surgery. Solid food was withheld for 12 hours and liquid food for four hours before surgery in all the cases. Ocular instillation with antibiotic, ciprofloxacin eye drops, at the rate of one drop four times daily was started prior to surgery.

On the day of surgery affected eye was thoroughly irrigated with normal saline solution and the periocular area was cleaned with sterile cotton to remove accumulated ocular discharge, dirt and tissue debris. The eyelashes were clipped close to palpebral border. The area around the eye was thoroughly scrubbed with povidone iodine solution. The eye was again irrigated with sterile normal saline and then instilled a few drops of povidone iodine[7] (5% w/v) ophthalmic solution.

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3. Sporidex DS, (250mg tablets), Ranbaxy laboratories Ltd. A.P.

4. Diamox, (250 mg tablets), Wyeth Ltd, Worli, Mumbai.

5. Dorzox eyedrops (2%), Cipla Ltd., Verna, Goa.

6. Glucomol eye drops (0.5%), Allergan India Ltd., Bangalore.

7. Apidine-5 (5% w/v), Appasamy Ocular Devices Pvt. Ltd, Pondichery

3.2.2. Preparation of collagen sheet

Collagen was used as a potential biomaterial for the repair of corneal lesions. The collagen sheet of bovine intestinal origin was procured from M/s Animal Byproducts Ltd. Chennai. It was prepared from the submucosal layer of intestine. Submucosal layer of intestine was separated by repeated scraping and washing of both sides and dried it. Collagen sheet (Fig.1) appears as semiluscent sheet when dry and preserved in air tight packets. When moistened the collagen sheet became more transparent and pliable (Fig. 2).

3.2.3. Anaesthesia

All the dogs were premedicated before the induction of general anaesthesia. Atropine sulphate[8] at the dose rate of 0.045 mg/kg body weight was administered intramuscularly followed by xylazine hydrochloride[9] at the rate of 1.5 mg/kg bodyweight. General anaesthesia was induced with ketamine hydrochloride[10] at the rate of 5 mg/kg given intramuscularly after fifteen minutes of xylazine administration for all the dogs in Group I and Group II.

Anaesthesia was maintained using a combination of equal volumes of xylazine and ketamine along with diazepam[11] at the rate of 0.5 mg/kg bodyweight administered intravenously 'to effect'.

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8. Atropine sulphate injection, (0.6 mg/ml), Mount Mettur Pharmaceuticals Ltd, India

9. Xylaxin, Indian Immunologicals Ltd (20mg/ml), Andrapradesh

10. Ketmin 50, Themis Medicare Ltd, Mumbai

11. Calmpose injection, Ranbaxy, New Delhi

3.2.4. Surgical Technique

The following surgical procedures were conducted in this study. Corneal debridement/scarification and / or superficial keratectomy were done in all the cases and application of collagen sheet was done in Group I. Temporary tarsorrhaphy was done in all the cases.

3.2.4.1. Corneal debridement

The dogs were positioned in lateral recumbency with affected eye above and were draped. The eyelids were kept retracted with the help of an eye speculum. Dry cotton tipped applicator was used to remove the abnormal epithelium and debris from the stromal surface. The fine corneal scissors (Fig.4) were used to facilitate the removal of loose epithelium. The defect was aggressively debrided until epithelium no longer dislodged easily from the stroma. The epithelium was removed about 1-2 mm away from the margin of corneal lesion (Moore, 2003).

3.2.4.2. Superficial keratectomy

The dog was positioned and the eye was prepared as above. A circumscribed incision around the lesion was made to the desired depth using the tip of a No. 11 Bard Parker blade. Then the edge of the incised cornea was grasped with fine forceps and elevated slightly. Either a Bard Parker No. 11 blade or the corneal scissors was used to continue the dissection in a horizontal plane. Stromal dissection was maintained in a single plane to create a smooth keratectomy bed and to avoid corneal perforation, by keeping the blade parallel to the corneal lamellae all the times (Hollingsworth, 2003).

3.2.4.3. Application of collagen sheet

The dog was positioned in lateral recumbency with affected eye above and was draped. The eyelids were kept retracted with the help of an eye

speculum. Collagen sheet was cut into the desired size and shape with scissors. It was made large enough to cover the cornea and was thoroughly washed with sterile normal saline. Then, it was dipped in gentamicin[12] eye drops (Fig. 3) for about 15 minutes and kept over the newly prepared corneal recipient bed. To ensure the retention of the collagen sheet in the eye temporary tarsorrhaphy using 1/0 braided silk sutures was performed.

3.2.4.4. Temporary tarsorrhaphy

In all the surgical cases, complete tarsorrhaphy was done to protect the cornea. One or two interrupted horizontal mattress sutures were used for temporary tarsorrhaphy. The 1/0 silk sutures were placed three millimeters from the upper eyelid margin with the distance of five millimeters between the sutures. Ocular instillation of eye drops were done through the small opening left at the medial canthus.

3.2.5. Postoperative Care

Elizabethan collar (Fig. 5) was advised in all the cases to prevent self mutilation, till complete healing. Parentral administration of ceftriaxone[13] at the rate of 20 mg/kg body weight was given in all the cases on the day of surgical intervention. Topical antibiotic and flurbiprofen were instilled four times daily in all cases.

If required, the topical administration of the ciprofloxacin was changed later based on the results of culture and sensitivity test of corneal swab. Oral antibiotics, cephalexin at the rate of 20 mg/kg body weight was given twice daily for five days and multivitamins were administered in all the cases. Oral administration of Acetazolamide, at the rate of 20 mg/kg body weight, or topical instillation of Timolol maleate and Dorzolamide were done when intraocular pressure in the opposite eye was found elevated.

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12. Gracin eye drops(0.3%), Biochem Pharmaceutical Industries Ltd., Mumbai

13. Intacef, (250 mg), Intas Pharmaceuticals Ltd, Ahmedabad, India

3.2.6. Complications

Post operative complications like infection, mutilation, pigmentation, corneal opacity and others, if any, were recorded in all the cases. Suitable modifications in the treatment were made accordingly.

3.3. MAIN ITEMS OF OBSERVATION

3.3.1. Physiological Parameters

The rate of respiration (per minute), pulse rate (per minute) and rectal temperature (^{o}C) were recorded in all cases.

3.3.2. Clinical Examination

3.3.2.1. General condition of patient

The general condition of all the animals were visually assessed and categorized as excellent, good, fair and poor.

3.3.2 2. Wet film Examination

One drop of blood was placed on a glass slide, covered with coverslip without air bubbles and observed under low power objective of microscope to identify the presence of any moving blood parasites and observations were recorded on the day of presentation.

3.3.2 3. Condition of Eye

The eye was observed for the presence and nature of ocular discharge, visual function, corneal clarity, corneal oedema, vascularization of cornea, type and extends of the lesion, fluorescein dye test, Schirmer's tear test and any other relevant observations.

3.3.2.3.1. Nature of discharge

Eyes were observed for the presence or absence of lacrimation or ocular discharge in all the cases. If present, the nature of the discharge was recorded as serous, mucoid or purulent.

3.3.2.3.2. Type and extend of the lesion

The type and extend of the lesions were assessed and categorized in to superficial / deep, descemetocele or staphyloma.

3.3.2.3.3. Visual function tests

The visual function status of the affected animals was assessed based on the manace reflex and pupillary reflex.

3.3.2.3.4. Corneal clarity

The clarity of cornea was assessed based on visual examination. The clarity was recorded as clear (4+), hazy (3+), moderate opacity (2+) and complete opacity (1+).

3.3.2.3.5. Corneal Oedema

The presence of corneal edema/ corneal opacity in all cases was recorded as present (+) or absent (-).

3.3.2.3.6. Vascularization of Cornea

All the affected eyes were examined for the presence of neovascularization of cornea and its changes till the end of the observation period and recorded.

3.3.2.3.7. Fluorescein dye test

The presence and extend of corneal ulcers were assessed by the use of fluorescein dye in the form of sterile impregnated strips (Fig.3). The strip was

inserted into the conjunctival sac and kept the lids closed for a few seconds. The dog was allowed to blink a few times to disperse the stain over the cornea. Afterwards the excess stain was flushed out with sterile normal saline. The cornea was stained fluorescent green wherever there was loss of epithelium but deep corneal defects that extended to the level of descemet's membrane did not stain.

3.3.2.3.8. Schirmer's tear test (STT)

Schirmer's tear test assesses the quantitative production of the aqueous portion of the tear film by the use of sterile Schirmer's tear test strips (Fig. 3) with a printed millimeter scale on it. The strip was placed into the ventral conjunctival cul-de-sacfor exactly one minute. Then the strip was removed and the distance of wetness is immediately measured in millimeters. The normal Schirmer's tear test in a dog ranges from 14 to 25 mm per minute.

3.3.2.3.9**.** Other relevant observations

Observations like increased intraocular pressure, hypopyon, hyphaema, corneal pigmentation and other specific lesions, if any, were recorded. Intraocular pressure of the opposite eye was measured using Schiotz tonometer.

3.3.3. Haematological Parameters

Blood samples were collected in EDTA and were used for haematological evaluation *viz*., haemoglobin concentration (Hb) (Sahli's acid haematin method), Erythrocyte sedimentation rate (ESR) (Wintrobe haematocrit method), Total Leucocyte count (TLC). Blood smears were prepared for differential leucocyte count (DLC) and the results were recorded.

3.3.4. Biochemical parameters

Blood glucose level was estimated in all the cases only on the day of presentation.

3.3.5. Culture and sensitivity test of corneal swab

The swabs for the culture and sensitivity test were collected before the instillation of any medication. The eyelids were gently retracted and the sides of the sterile swabs were rolled over the area of ulceration. Care was taken not to touch the eyelid margin or eyelashes. The samples were inoculated in Bovine Heart Infusion Agar using quadrant- streaking method. All the plates were incubated at 37°C for 24 hours and were examined for the presence of bacterial growth, if any. Sensitivity test was carried out by antibiogram.

The results were expressed as mean ± standard error. The values obtained were analysed by one way Analysis of Variance to determine the level of significance. The value of $P < 0.05$ was considered significant (Snedecor and Cochran, 1985).

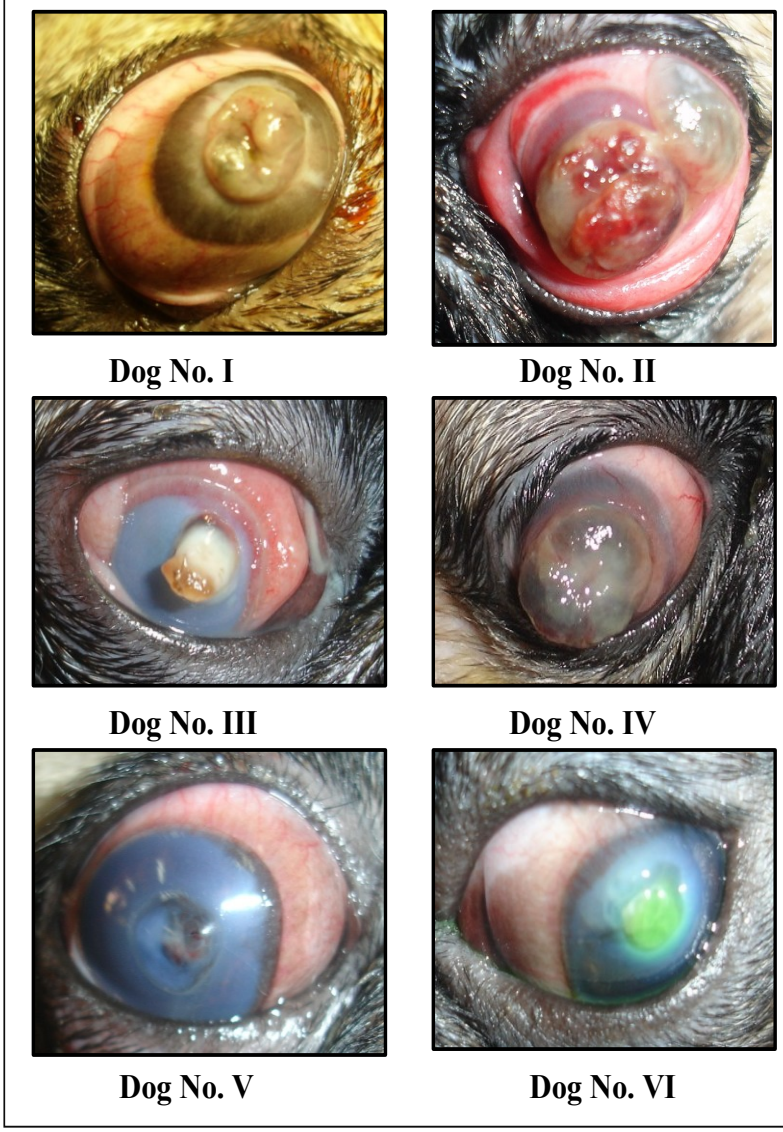

Plate 1. Eyes subjected to treatment in Group I

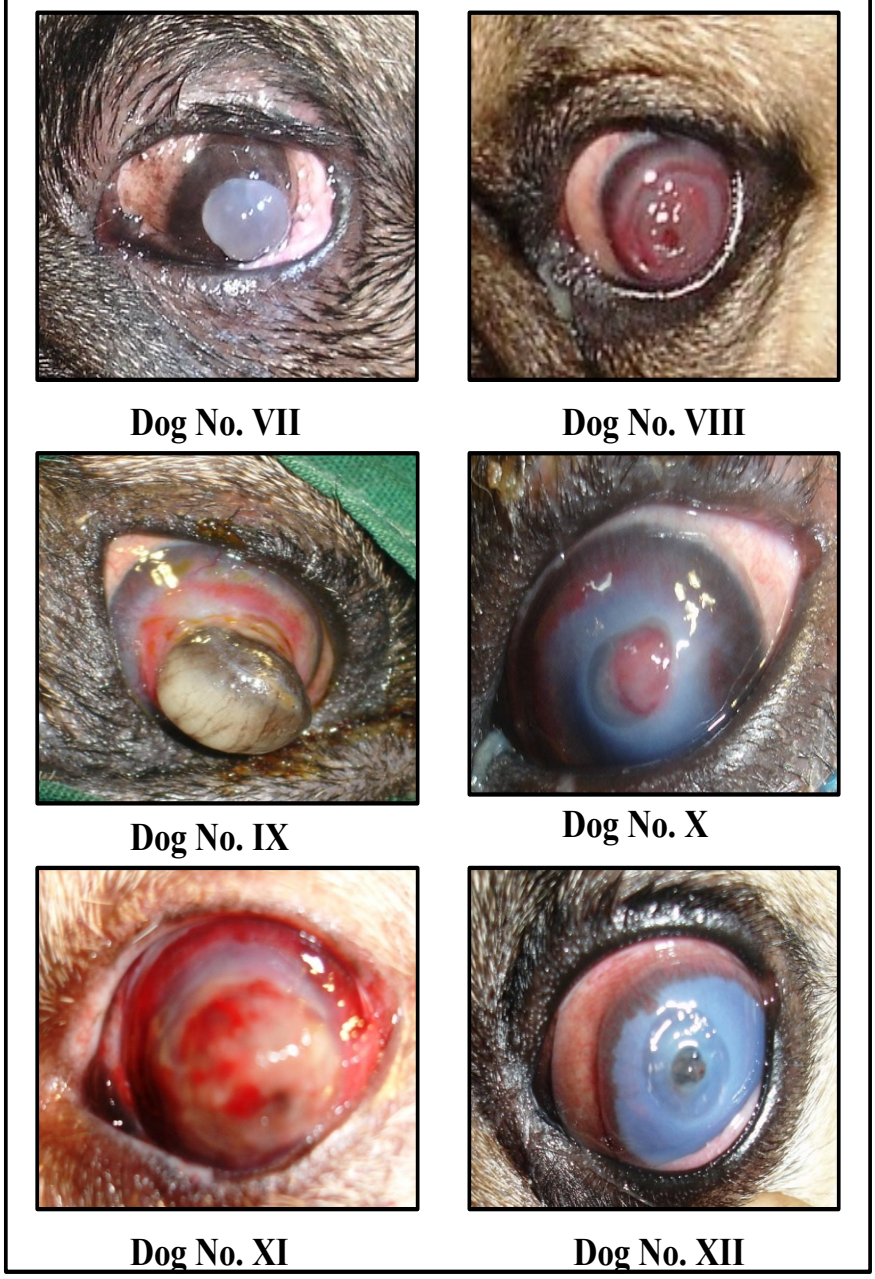

Plate 2. Eyes subjected to treatment in Group II

Fig.1. Collagen sheet before soaking in gentamicin

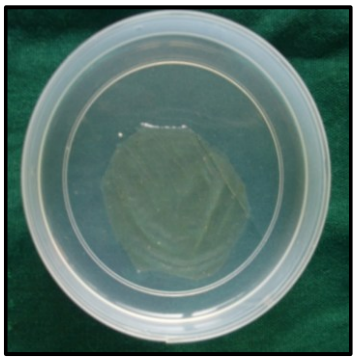

Fig. 2. Collagen sheet after soaking in gentamicin

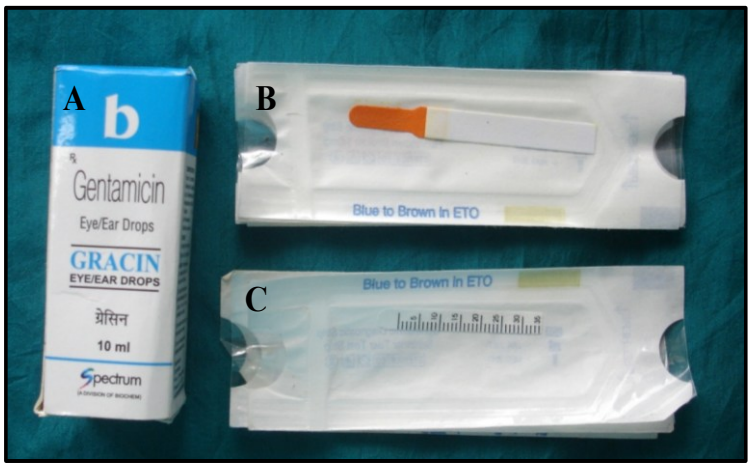

Fig. 3. Antibiotic (Gentamicin eye drops) used for soaking collagen sheet (A), Fluorescein dye strip (B) and Schirmer's tear test strip (C)

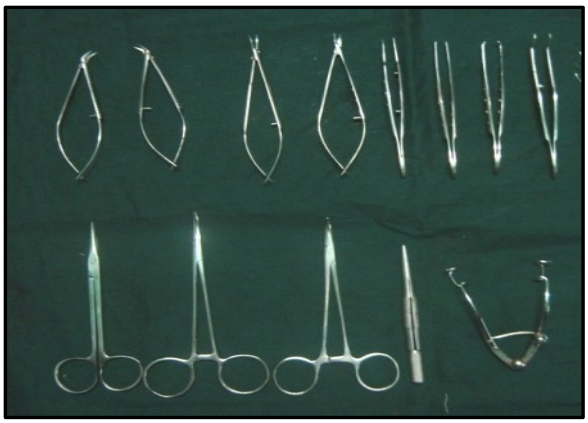

Fig.4. Ophthalmic instruments used for corneal surgery

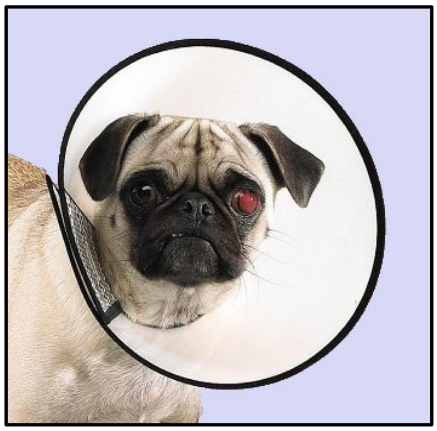

Fig. 5. Dog with Elizabethan collar

Results

4. RESULTS

4.1. SELECTION OF CASES

All the dogs presented to Veterinary College Hospitals at Mannuthy and Kokkalai with corneal ulceration were thoroughly examined, and twelve dogs were randomly selected for the study. All the cases were subjected to detailed clinical and ophthalmological examination.

Among the cases considered for the study, the lesions included eight cases of staphyloma (Dog Nos. I, II, III, IV, VII, IX, XI and XII) and four cases of deep stromal corneal ulcers (Dog Nos. V, VI, VIII and X). The exciting cause of lesion was not obvious in any of the cases except Dog No. XI, where the condition was caused by a road accident. In Dog Nos. I, II, V, VI, VII, VIII, IX and X the intraocular pressure of the other eye was highly increased and were treated for glaucoma.

In dogs presented with staphyloma, the protruding portions were snipped off and scarified the area. The iris was found adhered to the cornea in all these cases. Among the dogs, six dogs were included into Group I (Dog Nos. I, II, III, IV, V and VI) and were subjected to scarification / superficial keratectomy followed by application of collagen sheet over the cornea. The remaining six cases were included into Group II (Dog Nos. VII, VIII, IX, X, XI and XII) and were subjected to scarification or debridement or superficial keratectomy.

4.1.1. Signalment and Anamnesis (Table 1)

The age of dogs under this study ranged from one month to five years with a mean value of 13.58 ± 5.06 months. Out of the twelve cases, eleven were Chinese Pugs, and one was Mongrel dog (Fig. 6). Of the twelve cases seven were males and rest were females. Left eye was found affected in eight cases and right in four. According to the history revealed by the owners, illnesses were noticed on an average of 6.83 ± 1.39 days before presenting the cases to the hospitals.

Seven animals (Dog Nos. I, III, IV, VI, VII, IX and XII) had the history of previous medical treatment without much beneficial effect for the same condition. Dog No. I was under the treatment of fever and anorexia for the last two weeks and Dog No. VIII had the previous incidence of corneal ulcer in the other eye and medically treated. Dog No. VI was under medical treatment for corneal cloudiness for the last three months. The exciting cause was trauma in most cases due to the prominence of eyeballs, as per the history narrated by the owners.

4.2. SURGICAL TREATMENT

4.2.1. Preoperative Preparation

Clipped the eyelashes and hairs in the periocular area. Thoroughly irrigated the eye with normal saline solution in order to remove the dirt and debris. Scrubbed the adnexal areas with povidone iodine solution (1:50) and povidone iodine solution (5% w/v) was instilled in to the eye and were found to be satisfactory method of preparation of the site for surgery.

4.2.2. Application of collagen sheet

Collagen sheet of bovine intestinal origin became pliable and transparent after soaking in gentamicin eyedrops. Thus it enabled the easy placement over the newly prepared recipient bed on cornea. It covered corneal surface without the formation of air pockets and stick to it. Temporary tarsorrhaphy was found enough to retain the collagen sheet in position.

4.2.3. Anaesthesia

The anaesthetic regimen adopted was found satisfactory for the surgical manipulation in all the cases. Induction and recovery were smooth and uneventful.

4.2.4. Surgical Technique

In Dog Nos. I, II, III, IV, VII, IX, XI and XII corneal ulcers were complicated and resulted in staphyloma by the day of presentation to the hospital. In all these dogs, the protruded iris was covered with fibrin clot except in Dog No. XII. Fibrin clots were removed using corneal scissors. Iris in all these cases were severely adhered to the cornea and no seepage of the aqueous humor were noticed. As the adhesion between the iris and cornea were difficult to be separated, iridectomy was performed. Since the corneal wound edges were unable to appose by suturing, it was kept as such in all these dogs. The edges of the corneal wound was scarified with the tip of BP blade No. 11 (Fig. 7).

Dog No. V and VI in Group I were subjected to scarification of the ulcer and superficial keratectomy.

All the dogs in Group I after the correction of staphyloma and scarification were followed by application of collagen sheet to cover the corneal defect (Fig. 8). There was no difficulty in fixing the collagen sheet and were retained in position by tarsorrhaphy.

The dogs in Group II were subjected scarification of corneal ulcer after correction of staphyloma except in Dog No. VIII and X, where scarification and superficial keratectomy were performed.

Temporary tarsorrhaphy done was effective in protecting the eye and for retaining the collagen sheet (Fig.9). Partial disruption of tarsorrhaphy sutures was noticed in Dog Nos.III and VIII. The sutures in all other cases were intact and were removed on 7^{th} postoperative day.

4.2.5. Post Operative Care

All the dogs were provided with Elizabethan collar during the post operative period in order to prevent self mutilation. In all the cases ceftriaxone

injection was administered at the rate of 20 mg/kg body weight on the day of surgery. In order to counter act infection and inflammation topically ciprofloxacin and flurbiprofen were instilled at an interval of 10 minutes four times daily till complete healing. Topical medications in all dogs were done efficiently through the space in medial canthus up to seventh post operative day. Cephalexin was given orally at the dose rate of 20 mg/kg body weight twice daily for the first five days. All the animals tolerated the medication and it was successful in controlling the postoperative infection and pain.

Increased intraocular pressure was noticed in the opposite eye in Dog Nos. I, II, V, VI, VII, VIII, IX and X and was treated with oral administration of Acetazolamide (Carbonic anhydrase inhibitor) and topical administration of dorzolamide in Dog No. II and timolol maleate (nonselective beta blocker) in rest. It was effective in reducing the intraocular pressure and controlling the Glaucoma.

4.3. MAIN ITEMS OF OBSERVATIONS

4.3.1. Physiological parameters (Table 2)

4.3.1.1. Rate of Respiration

The mean rate of respiration (per minute) was 31.00 ± 1.71 on the day of presentation. It was 28.83 ± 1.37, 27.17 ± 0.91, 26.17 ± 1.04, 27.50 ± 0.61 and 27.17 ± 0.74 on 7^{th}, 14^{th}, 28th, 45^{th} and 60^{th} day respectively in Group I and all the values were within normal range

The mean rate of respiration (per minute) was 33.33 ± 1.97 on the day of presentation. It was 30.83 ± 1.22, 32.67 ± 2.76, 30.33 ± 2.84, 29.50 ± 1.40 and 28.00 ± 0.73 on 7^{th}, 14^{th}, 28th, 45^{th} and 60^{th} day respectively in Group II and all the values were within normal range.

4.3.1.2. Rectal Temperature

The mean rectal temperature (°C) in Group I dogs were 38.83 ± 0.29, 38.80 ± 0.10, 38.77 ± 0.07, 38.89 ± 0.07, 38.88 ± 0.02 and38.96 ± 0.05 on the day of presentation, on 7th, 14th, 28th, 45th and 60th day respectively. All the values were within normal range.

The mean rectal temperature (°C) in Group II dogs were 38.96 ± 0.20, 38.89 ± 0.16, 38.65 ± 0.09, 38.83 ± 0.08, 38.75 ± 0.06 and 38.88 ± 0.06 on the day of presentation, on 7th, 14th, 28th, 45th and 60th day respectively. All the values were within normal range.

4.3.1.3. Pulse Rate

The mean pulse rate (per minute) recorded on the day of presentation was 98.33 ± 4.04 and on 7th, 14th, 28th, 45th and 60th day the average pulse rate were 96.67 ± 3.45, 90.50 ± 2.18, 91.00 ± 2.23, 91.00 ± 1.34 and 94.33 ± 2.21 respectively in Group I. All the values were within normal range.

The mean pulse rate (per minute) recorded on the day of presentation was 91.00 ± 5.05 and on 7th, 14th, 28th, 45th and 60th day the average pulse rate were 91.00 ± 5.36, 91.00 ± 6.38, 84.33 ± 4.11, 90.67 ± 1.60 and 88.33 ± 2.02 respectively in Group II and all the values were within normal range.

4.3.2. Clinical observations

4.3.2.1. General Condition of Patient

All the dogs, among both the Group were found to have a general condition scored good except in Dog No. I, which had poor condition on the first day and recovered good health in later observations.

4.3.2.2. Wet Film Examination

Moving blood parasites were not detected preoperatively in any of the dogs.

4.3.2.3. Condition of the Eye

4.3.2.3.1. Nature of discharge

Nature of the discharge was purulent in all the dogs in Group I except Dog Nos. V and VI on the first day of observation. It became clear by the 7th day of observation. Except Dog No. X, all the cases in Group II were presented with purulent discharge on the first day of observation and that became clear on 14th day of observation. In all the cases the discharges were clear in the later observation period.

4.3.2.3.2. Type and extend of lesion

In Group I, Dog Nos. I, II and IV were presented with complete damage of cornea and with prolapse of the iris resulted in the collapse of anterior chamber. In Dog No. III corneal perforation occurred and complicated by the formation of staphyloma which was covered with fibrin and debris. Dog Nos. V and VI were presented with deep stromal corneal ulceration.

The collagen sheet applied over the cornea after scarification, superficial keratectomy and correction of staphyloma in all these cases were completely dissolved by third postoperative day.

In Dog No. I collagen sheet was completely dissolved by 3rd postoperative day (Fig.10) and the corneal defect became shallow by 7th observation day (Fig. 11). By 28th day the defect was completely covered and the center of the lesion remained opaque (Fig.12).

In Dog No. II and III the center of the lesion became shallow on 7th day and by 28th day cornea regained the normal shape.

The cornea of Dog No. IV was covered but with a small facet in the centre of the lesion by 28th day and completely regained the shape by 60th day.

In Dog No. V and VI the stromal ulcers were covered completely by 28th day and the normal cornea were regained by 60th day of observation.

In Group II, cornea of Dog Nos. VII, IX and XII were ruptured and resulted in staphyloma on the day of presentation. In which Dog Nos. VII and IX were more complicated and anterior chamber was already collapsed.

In Dog No. VIII cornea was completely damaged but did not penetrate the full depth and stromal ulceration was stationary in nature. The lesion was started to cover by 14th day and completed by 60th day of observation but the central opacity persisted.

The size and depth of the lesion in Dog Nos. X (Fig. 15) and XII were reduced progressively and completely covered by 45th day of observation.

Cornea was completely damaged and infected in Dog No. XI and iris were covered by the exudates and fibrin clots. Corneal covering was observed by 14th day and regained its shape by 28th day. By 45th day cornea was reconstituted but clarity was completely lost.

4.3.2.3.3. Visual function evaluation

In all the cases visual reflexes could not be assessed due to severity of corneal damage and also due to corneal oedema and opacity. In all the cases except Dog Nos.V,VI,X and XII, cornea was completely damaged and vision was compromised. By 45th postoperative day menace reflex was observed in Dog Nos.V and VI.

Menace reflex was observed in Dog.No.X by the end of observation

period.In Dog No. XII normal menace and pupillary reflexes were observed on 45th day.

4.3.2.3.4. Corneal clarity (Table 3 and 4)

In Group I, Dog Nos. III and V were presented with moderate opacity (2+) and all others were presented with complete opacity (1+) on the first day.

In Dog Nos. I, II and IV cornea was completely perforated and covered with fibrin and blood clots. In these dogs corneal lesions were covered by 7th day and were opaque.

In Dog No. I, a progressive reduction in the area of corneal opacity noticed and the central opacity persisted till the end of observation period (Fig.13).

By 45th day, the area of corneal opacity was reduced in Dog No.II but the central portion became completely opaque and it remained unchanged in later observations.

In Dog No. III, moderate opacity of the cornea persisted till 28th day of observation and afterwards central haziness was observed.

In Dog No. IV the cornea became moderately opaque by 28th day and opacity concentrated to the center of the lesion by 60th day (Fig.23).

In Dog No. V the moderate opacity was retained till 28th day and became clear by 60th day (Fig. 19).

The periphery of cornea became clear with central haziness by 45th day of presentation in Dog No. VI (Fig. 20) and completely cleared by the end of observation period.

All dogs except Dog Nos.V and VI in Group I, corneal pigmentation was observed on the periphery of the lesion.

In Group II, all the dogs were presented with complete opacity of the cornea with staphyloma in Dog No. VII, IX, XI and XII.

Cornea was completely damaged and opaque in Dog Nos. VII and XI on the day of presentation. Corneal damage was covered but the central opacity persisted on subsequent observations (Fig. 25).

Corneal opacity of the Dog No. VIII was persisted till 45th day and became moderately opaque by the end of observation period.

In Dog No. IX, cornea became moderately opaque by 45th day and remained as such till the end of observation period.

In Dog No. X, corneal opacity was progressively reduced to a state of moderate opacity by 28th day onwards (Fig. 16) and the cleared cornea with central haziness was observed till the end of the observation (Fig.17).

In Dog No. XII, corneal haziness at the center of the lesion was observed on 28th day onwards (Fig. No. 21) and became clear by 60th day, but with formation of anterior synechia.

Corneal pigmentation was observed in all dogs in Group II except Dog No.XII.

4.3.2.3.5. Corneal oedema (Table 5 and 6)

Corneal oedema was observed in all the dogs under this study and a progressive reduction was there in all cases during the subsequent observation periods. In Group I, all the dogs were presented with corneal oedema on the day of presentation which persisted till 14th day of observation. In Dog No. III, IV, V and VI, the oedema was reduced but present on 28th day of observation. On subsequent observations oedema was absent in all the cases.

In Group II, corneal oedema persisted throughout the observation period in Dog No. VII. Corneal oedema in Dog Nos. VIII and IX were observed up to 45th day of observation and in Dog Nos. X and XI, it persisted up to 28th day. In Dog No. XII, it was observed up to 14th postoperative day.

4.3.2.3.6. Vascularization of cornea (Table 7 and 8)

In Group I, vascularization of the cornea was observed in four dogs *viz.* Dog Nos. I, II, III and IV on the day of presentation itself and it was found increasing on seventh postoperative day. Vascularization of cornea was observed from seventh day onwards and degree of neovascularization increased up to fourteenth day in Dog Nos. V (Fig. 18) and VI. Regression of blood vessels was observed in later observation periods and complete resolution by 28th day except in Dog No. V, where it occurred by 45th day.

Dog Nos. VIII, IX and XI in Group II were presented with neovascularization of the cornea and its intensity increased on 7th day of observation. Vascularization in them persisted till 28th day (Fig.22) of observation and become negligible on 45th day of observation.

In Dog Nos. VII, X and XII vascularity of cornea was observed by 7th day and remained till 14th day of observation and the cornea was devoid of any blood vessels in later observation periods.

4.3.2.3.7. Fluorescein Dye Test (Table 9 and 10)

In Group I, fluorescein dye test was positive in all the dogs on the first day of presentation. By 3rd day, the test became negative in Dog Nos. I, V and VI. Flourescein test became negative on 5th postoperative day in Dog No. III. In all the dogs except Dog No. IV this test became negative on 7th postoperative day. In Dog No. IV dye retention was observed on the centre of the lesion on 7th day and

became negative on later observations.

In Group II fluorescein dye retention was observed in all the dogs till 14th day except Dog No. X (Fig.14) where it was observed till 7th day of observation. In Dog Nos. VIII and IX, fluorescein dye retention was observed on 28th day of observation and was absent in all later observations.

4.3.2.3.8. Schirmer's tear test (Table11)

The mean values (mm/min) were 18.00 ± 2.08, 19.67 ± 1.43, 20.67 ± 1.08, 21.50 ± 0.61, 23.17 ± 0.54 and 23.50 ± 0.84 on the day of presentation, 7th, 14th, 28th, 45th and 60th day respectively in Group I and the values were within the normal range.

The mean values (mm/min) were 23.00 ± 1.00, 23.00 ± 1.50, 22.50 ± 1.25, 21.83 ± 0.65, 21.33 ± 0.95 and 21.83 ± 1.40 on the day of presentation, 7th, 14th, 28th, 45th and 60th day respectively in Group II and the values were within the normal range.

4.3.2.3.9. Other relevant observations

In Group I, increased intraocular pressure was observed in Dog Nos. I, II, V and VI and the values in millimeters of Hg were 46.3, 41.6, 43.4 and 38.3 respectively. Beneficial reductions in the intraocular pressure were obtained by the treatment with acetazolamide tablets (Dog No. I, V and VI) and dorzolamide eyedrops (Dog No. II). In Case No. V acetazolamide tablets were stopped after 1 week because of the respiratory distress and timolol maleate eyedrops were advised.

In Group II, elevated intraocular pressure was observed in Dog Nos. VII, VIII, IX and X and the values in millimeters of Hg were 59.4, 46.7, 49.3 and 40.4 respectively. Conditions were managed successfully with oral administration of

acetazolamide tablets in all the cases. In Dog No. VIII and IX in addition to it, timolol maleate eyedrops were also applied.

Corneal pigmentation was observed in most of the cases under this study except Dog Nos. V, VI and XII. Pigmentation of the cornea was developed in the limbal margin of Dog Nos. I, II, IV, VII, VIII, IX and X on the day of presentation itself. It resulted in the loss of corneal clarity in the peripheral area.

4.3.3. Haematological Parameters

4.3.3.1. Haemoglobin Concentration (Table 12)

In Group I dogs, the mean values of haemoglobin concentration (g/dl) were 12.30 ± 0.38, 12.20 ± 0.37, 12.33 ± 0.22, 12.40 ± 0.24 and 13.13 ± 0.13 on the day of presentation, 7^{th}, $28^{th,}$ 45^{th} and 60^{th} day respectively and all the values were within normal range.

In Group II dogs, the mean values of haemoglobin concentration (g/dl) were 11.80 ± 0.67, 13.03 ± 0.30, 12.66 ± 0.18, 12.55 ± 0.32 and 13.46 ± 0.25 on the day of presentation, 7^{th}, $28^{th,}$ 45^{th} and 60^{th} day respectively and all the values were within normal range.

4.3.3.2. Erythrocyte Sedimentation Rate (Table 12)

The mean values (mm/hour) were 8.56 ± 0.56, 9.26 ± 0.66, 10.36 ± 0.40 10.76 ± 0.28, and 11.03 ± 0.12 on the day of presentation, 7^{th}, 28^{th}, 45^{th} and 60^{th} day respectively in Group I and the values were observed within normal range.

The mean values (mm/hour) were 9.00 ± 0.72, 9.40 ± 0.42, 10.13 ± 0.25, 10.76 ± 0.30 and 10.80 ± 0.23 on the day of presentation, 7^{th}, 28^{th}, 45^{th} and 60^{th} day respectively in Group II and the values were observed within normal range.

4.3.3.3. Total Leukocyte Count (Table 12)

The mean values of total leukocyte count (x 10^3 cells/ cmm) were 10.85 ± 0.28, 11.06 ± 0.24, 11.56 ± 0.26, 11.17 ± 0.22 and 11.24 ± 0.28 on the day of presentation, 7^{th}, 28^{th}, 45^{th} and 60^{th} day respectively in Group I and all the values were within normal range.

The mean values of total leukocyte count (x 10^3 cells/ cmm) were 10.75 ± 0.22, 11.28 ± 0.29, 11.67 ± 0.21, 11.54 ± 0.18 and 11.65 ± 0.26 on the day of presentation, 7^{th}, 28^{th}, 45^{th} and 60^{th} day respectively in Group II and all the values were within normal range.

4.3.3.4. Differential Leukocyte Count (Table 13)

The mean differential count of neutrophils (%) in Group I was 75.67 ± 1.43, 74.67 ± 1.08, 76.00 ± 0.57, 74.50 ± 0.61 and 76.17 ± 1.44 on the day of presentation, 7^{th}, 28^{th}, 45^{th} and 60^{th} day respectively. The mean differential count of neutrophils (%) in Group II was 74.83 ± 1.07, 74.00 ± 0.96, 75.67 ± 1.25, 74.83 ± 0.87 and 76.33 ± 0.98 on the day of presentation, 7^{th}, 28^{th}, 45^{th} and 60^{th} day respectively

The mean percentage for lymphocytes in Group I was 21.83 ± 1.13, 21.50 ± 0.84, 21.00 ± 0.51, 22.17 ± 0.60 and 20.67 ± 1.08 on the day of presentation, 7^{th}, $28^{th,}$ 45^{th} and 60^{th} day respectively. The mean percentage for lymphocytes in Group II was 21.50 ± 0.71, 22.50 ± 0.76, 21.00 ± 0.85, 22.17 ± 0.98 and 20.50 ± 0.88 on the day of presentation, 7^{th}, $28^{th,}$ 45^{th} and 60^{th} day respectively.

The average differential count for eosinophils (%) was 1.83 ± 0.40, 2.83 ± 0.47, 2.17 ± 0.16, 2.17 ± 0.30 and 2.33 ± 0.21 on the day of presentation, 7^{th}, 28^{th}, 45^{th} and 60^{th} day respectively in Group I. The average differential count for eosinophils (%) was 2.67 ± 0.33, 2.67 ± 0.33, 2.67 ± 0.49, 1.83 ± 0.30 and 2.17 ±

0.30 on the day of presentation, 7th, 28th, 45th and 60th day respectively in Group II.

In Group I the mean percentage of monocytes was 0.67 ± 0.21, 1.00 ± 0.25, 0.83 ± 0.30, 1.17 ± 0.30 and 0.83 ± 0.40 on the day of presentation, 7th, 28th, 45th and 60th day respectively. The mean percentage of monocytes in Group II was 1.00 ± 0.25, 0.83 ± 0.30, 0.67 ± 0.21, 1.17 ± 0.30 and 1.00 ± 0.25 on the day of presentation, 7th, 28th, 45th and 60th day respectively. Basophils were absent in all the samples examined.

All the values for differential leukocyte count remained within normal range and the values do not significantly differ within the groups.

4.3.4. Blood glucose level (Table 14)

The mean blood glucose levels (mg/dl) on the day of presentation were 91.17 ± 4.19 and 93.50 ± 2.36 in Group I and Group II respectively. All the values were within normal range.

4.3.5. Culture and sensitivity test (Table 15)

In Dog Nos. III, VI, VII, IX and XII no growth was obtained after incubation for 24 hrs. Gram positive cocci were isolated in Dog Nos. I, II, IV, V and X. Gram positive coccobacilli were isolated in Dog No. VIII. Gram positive cocci and Gram negative bacilli were concurrently isolated in Dog No. XI. In all the cases primarily selected ciprofloxacin was continued for the therapy based on the sensitivity results except in Dog No. X, where chloramphenicol was selected for the topical therapy. In Dog Nos. II, V and XI the isolated organisms were sensitive to enrofloxacin. Since ciprofloxacin was belongs to the same group the antibiotic was not changed.

Table 1: Anamnesis of dogs subjected for the study

Dog No.	Breed	Age (Months)	Sex	Condition of the eye	Eye affected	Duration of illness (Days)	Previous medication
I	Pug	9	Male	Staphyloma	Left	7	Gentamicin eye drops
II	Pug	1	Male	Staphyloma	Left	9	Nil
III	Pug	2	Male	Staphyloma	Right	5	Gentamicin and Dexachlor eyedrops
IV	Pug	1	Male	Staphyloma	Right	10	Chloromycetin and Diclofenac eyedrops
V	Pug	17	Female	Stromal corneal ulcer	Right	7	Nil
VI	Pug	3	Male	Stromal corneal ulcer	Left	2	Ofloxacin eyedrops
VII	Pug	36	Male	Staphyloma	Left	20	Pychlor drops
VIII	Pug	5	Female	Stromal corneal ulcer	Left	7	Nil
IX	Pug	9	Female	Staphyloma	Right	2	Pychlor eye drops
X	Pug	8	Female	Stromal corneal ulcer	Left	5	Nil
XI	Mongrel	60	Male	Staphyloma	Left	4	Nil
XII	Pug	12	Female	Staphyloma	Left	4	Gatifloxacin and Chlor-amphenicol eyedrops
Mean ± SE		13.58±5.06				6.83±1.39	

Table 2: Table showing mean values of physiological parameters

Parameters	Group	Days of observation					
		0th	**7th**	**14th**	**28th**	**45th**	**60th**
Rate of respiration (per minute)	**Group I**	31.00[a]±1.71	28.83[a]±1.37	27.17[a]±0.91	26.17[a]±1.04	27.50[a]±0.61	27.17[a]±0.74
	Group II	33.33[a]±1.97	30.83[a]±1.22	32.67[a]±2.76	30.33[a]±2.84	29.50[a]±1.40	28.00[a]±0.73
Rectal temperature (ºC)	**Group I**	38.83[a]±0.29	38.80[a]±0.10	38.77[a]±0.07	38.89[a]±0.07	38.88[a]±0.02	38.96[a]±0.05
	Group II	38.96[a]±0.20	38.89[a]±0.16	38.65[a]±0.09	38.83[a]±0.08	38.75[a]±0.06	38.88[a]±0.06
Pulse rate (per minute)	**Group I**	98.33[a]±4.04	96.67[a]±3.45	90.50[a]±2.18	91.00[a]±2.23	91.00[a]±1.34	94.33[a]±2.21
	Group II	91.00[a]±5.05	91.00[a]±5.36	91.00[a]±6.38	84.33[a]±4.11	90.67[a]±1.60	88.33[a]±2.02

Means bearing same superscript in the column do not differ significantly ($P > 0.05$).

Table 3: Observations on the degree of corneal clarity in Group I

Dog No.	Day of observation					
	0th	**7th**	**14th**	**28th**	**45th**	**60th**
I	1+	1+	1+	1+	1+	1+
II	1+	1+	2+	2+	1+	1+
III	2+	2+	2+	2+	3+	3+
IV	1+	1+	1+	2+	2+	2+
V	2+	2+	2+	2+	3+	4+
VI	1+	2+	3+	3+	3+	4+

Table 4: Observations on the degree of corneal clarity in Group II

Dog No.	Day of observation					
	0th	**7th**	**14th**	**28th**	**45th**	**60th**
VII	1+	1+	1+	1+	1+	1+
VIII	1+	1+	1+	1+	1+	2+
IX	1+	1+	1+	1+	2+	2+
X	1+	1+	1+	2+	2+	3+
XI	1+	1+	1+	1+	1+	1+
XII	1+	1+	2+	3+	3+	4+

1+ Complete opacity
2+ Moderate opacity
3+ Hazy
4+ Clear

Table 5: Observations on the degree of corneal oedema in Group I

Dog No.	Day of observation					
	0th	7th	14th	28th	45th	60th
I	+	+	+	_	_	_
II	+	+	+	_	_	_
III	+	+	+	+	_	_
IV	+	+	+	+	_	_
V	+	+	+	+	_	_
VI	+	+	+	+	_	_

Table 6: Observations on the degree of corneal oedema in Group II

Dog No.	Day of observation					
	0th	7th	14th	28th	45th	60th
VII	+	+	+	+	+	+
VIII	+	+	+	+	+	_
IX	+	+	+	+	+	_
X	+	+	+	+	_	_
XI	+	+	+	+	_	_
XII	+	+	+	_	_	_

\+ Present _ Absent

Table 7: Observations on the vascularization of cornea in Group I

Dog No.	Day of observation					
	0th	7th	14th	28th	45th	60th
I	+	+	+	_	_	_
II	+	+	+	_	_	_
III	+	+	_	_	_	_
IV	+	+	+	_	_	_
V	_	+	+	+	_	_
VI	_	+	+	_	_	_

Table 8: Observations on the vascularization of cornea in Group II

Dog No.	Day of observation					
	0th	7th	14th	28th	45th	60th
VII	_	+	+	_	_	_
VIII	+	+	+	+	+	_
IX	+	+	+	+	+	_
X	_	+	+	_	_	_
XI	+	+	+	+	+	_
XII	_	+	+	_	_	_

\+ Present _ Absent

Table 9: Observations on the fluorescein dye retention in cornea in Group I

Dog No.	Days of observation					
	0th	7th	14th	28th	45th	60th
I	+	_	_	_	_	_
II	+	_	_	_	_	_
III	+	_	_	_	_	_
IV	+	+	_	_	_	_
V	+	_	_	_	_	_
VI	+	_	_	_	_	_

Table 10: Observations on the fluorescein dye retention in cornea in Group II

Dog No.	Days of observation					
	0th	7th	14th	28th	45th	60th
VII	+	+	+	_	_	_
VIII	+	+	+	+	_	_
IX	+	+	+	+	_	_
X	+	+	_	_	_	_
XI	+	+	+	_	_	_
XII	+	+	+	_	_	_

\+ Present _ Absent

Table 11: Table showing mean values of Schirmer's Tear Test (mm/min)

Parameter	Group	Day of observation					
		0th	**7th**	**14th**	**28th**	**45th**	**60th**
STT (mm/min)	**Group I**	18.00[a]±2.08	19.67[a]±1.43	20.67[a]±1.08	21.50[a]±0.61	23.17[a]±0.54	23.50[a]±0.84
	Group II	23.00[a]±1.00	23.00[a]±1.50	22.50[a]±1.25	21.83[a]±0.65	21.33[a]±0.95	21.83[a]±1.40

Means bearing same superscript in the column do not differ significantly ($P > 0.05$).

Table 12: Observations on mean values of haematological parameters (A)

Parameters	Group	Day of Observation				
		0th	**7th**	**28th**	**45th**	**60th**
Hb (g/dl)	**Group I**	12.30[a]±0.38	12.20[a]±0.37	12.33[a]±0.22	12.40[a]±0.24	13.13[a]±0.13
	Group II	11.80[a]±0.67	13.03[a]±0.30	12.66[a]±0.18	12.55[a]±0.32	13.46[a]±0.25
ESR (mm/hr)	**Group I**	8.56[a]±0.56	9.26[a]±0.66	10.36[a]±0.40	10.76[a]±0.28	11.03[a]±0.12
	Group II	9.00[a]±0.72	9.40[a]±0.42	10.13[a]±0.25	10.76[a]±0.30	10.80[a]±0.23
TLC(x 0^3/cmm)	**Group I**	10.85[a]±0.28	11.06[a]±0.24	11.56[a]±0.26	11.17[a]±0.22	11.24[a]±0.28
	Group II	10.75[a]±0.22	11.28[a]±0.29	11.67[a]±0.21	11.54[a]±0.18	11.65[a]±0.26

Means bearing same superscript in the column do not differ significantly (P > 0.05).

Table 13: Observations on mean values of haematological parameter (B)

Parameters		Group	Day of Observation				
			0th	7th	28th	45th	60th
DLC (%)	N	Group I	75.67^{a}±1.43	74.67^{a}±1.08	76.00^{a}±0.57	74.50^{a}±0.61	76.17^{a}±1.44
		Group II	74.83^{a}±1.07	74.00^{a}±0.96	75.67^{a}±1.25	74.83^{a}±0.87	76.33^{a}±0.98
	L	Group I	21.83^{a}±1.13	21.50^{a}±0.84	21.00^{a}±0.51	22.17^{a}±0.60	20.67^{a}±1.08
		Group II	21.50^{a}±0.71	22.50^{a}±0.76	21.00^{a}±0.85	22.17^{a}±0.98	20.50^{a}±0.88
	E	Group I	1.83^{a}±0.40	2.83^{a}±0.47	2.17^{a}±0.16	2.17^{a}±0.30	2.33^{a}±0.21
		Group II	2.67^{a}±0.33	2.67^{a}±0.33	2.67^{a}±0.49	1.83±0.30	2.17^{a}±0.30
	M	Group I	0.67^{a}±0.21	1.00^{a}±0.25	0.83^{a}±0.30	1.17^{a}±0.30	0.83^{a}±0.40
		Group II	1.00^{a}±0.25	0.83^{a}±0.30	0.67^{a}±0.21	1.17^{a}±0.30	1.00^{a}±0.25
	B	Group I	0	0	0	0	0
		Group II	0	0	0	0	0

Means bearing same superscript in the column do not differ significantly (P > 0.05).

Table 14: Observations on blood glucose level on the day of presentation.

Group I		Group II	
Dog No.	**BGL (mg/dl)**	**Dog No.**	**BGL (mg/dl)**
I	81	VII	102
II	83	VIII	87
III	108	IX	92
IV	91	X	88
V	86	XI	94
VI	98	XII	98
Mean±SE	91.17^{a}±4.19	**Mean±SE**	93.50^{a}±2.36

Means bearing same superscript do not differ significantly (P > 0.05).

Table 15: Culture and sensitivity of the corneal swabs in the dogs studied

Dog No.	Bacterial growth	Sensitive	Resistant
I	Gram positive cocci	Ciprofloxacin++ Cefotaxim++ Chloramphenicol++ Enrofloxacin++	Amoxycillin Gentamicin
II	Gram positive cocci	Chloramphenicol+++ Enrofloxacin+++ Cefotaxim+++ Gentamicin++	Sulphadiazine
III	No Growth	_	_
IV	Gram positive cocci	Gentamicin +++ Ciprofloxacin+++ Cefotaxim++ Gatifloxacin++	Amoxycillin
V	Gram positive cocci	Gentamicin ++ Enrofloxacin++	Chloramphenicol Cefotaxim Penicillin
VI	No Growth	_	_
VII	No Growth	_	_
VIII	Gram positive coccobacilli	Ciprofloxacin ++++ Gentamicin++ Cefotaxim++	Sulphadiazine Amoxycillin
IX	No Growth	_	_
X	Gram positive cocci	Chloramphenicol+++ Gentamicin++ Cefotaxim++	Amoxycillin Ciprofloxacin
XI	Gram positive cocci and Gram negative rods	Chloramphenicol+++ Enrofloxacin+++ Streptomycin++ Cefotaxim++	Gentamicin
XII	No Growth	_	_

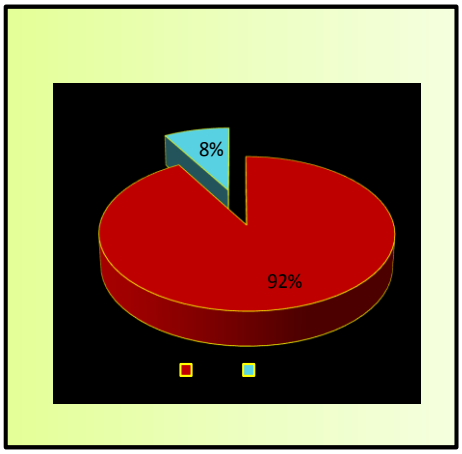

Fig. 6. Breedwise distribution of the corneal lesions under the study

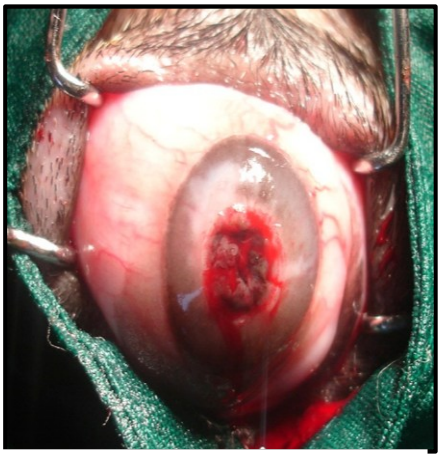

Fig. 7. After iridectomy and scarification (Dog No. I)

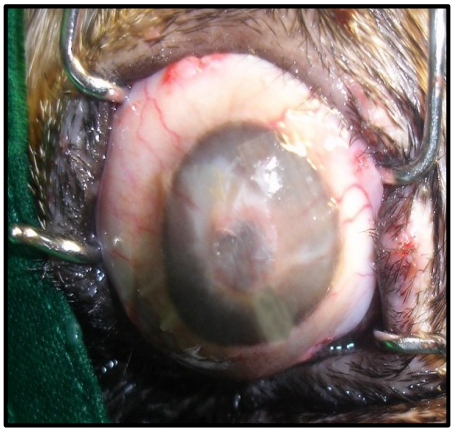

Fig. 8. After placing the collagen sheet (Dog No. I)

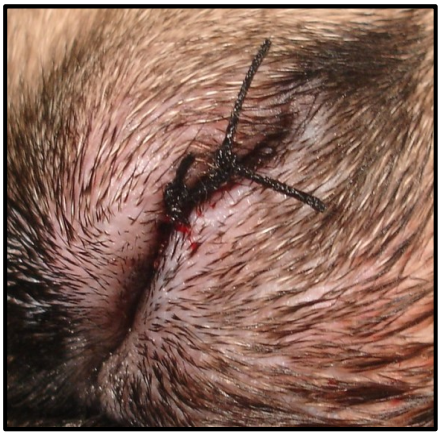

Fig.9. Temporary tarsorrhaphy (Dog No. I)

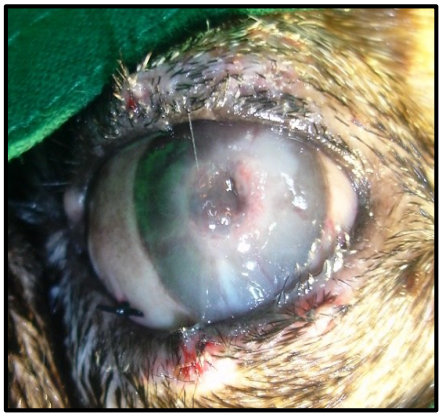

Fig. 10. Completely dissolved collagen sheet on 3rd day (Dog No. I)

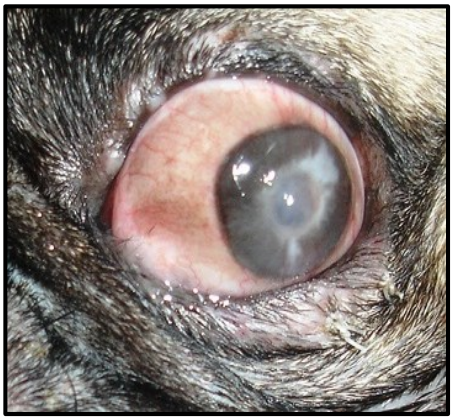

Fig. 11. Shallow corneal defect on 7th day (Dog No. I)

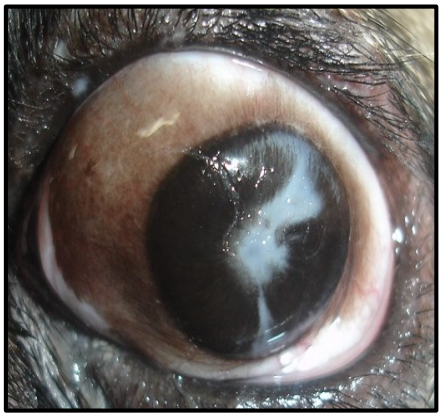

Fig. 12. Reconstructed cornea with central opacity and corneal pigmentation on 28th day (Dog No. I)

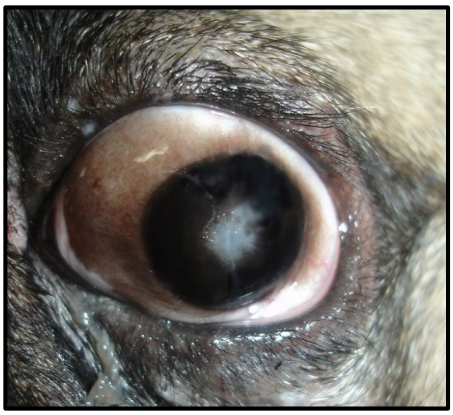

Fig. 13. Reduced central opacity on 60th day (Dog No. I)

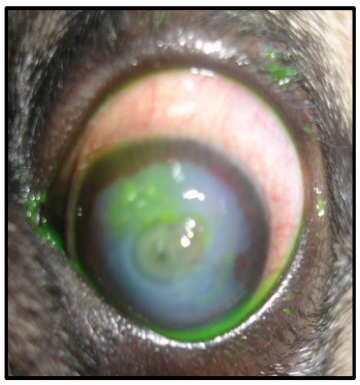

Fig. 14. Retention of fluorescein dye on 7th day (Dog No. X)

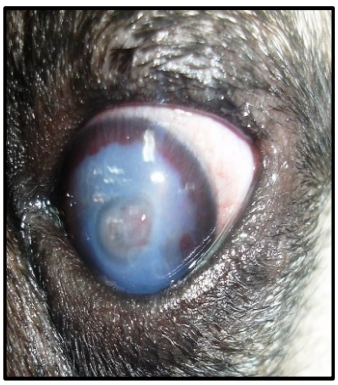

Fig. 15. Reduction in depth of corneal ulcer on 21st day (Dog No. X)

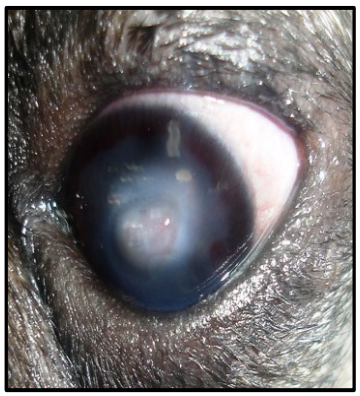

Fig. 16. Reduction in corneal oedema and opacity on 45th day (Dog No. X)

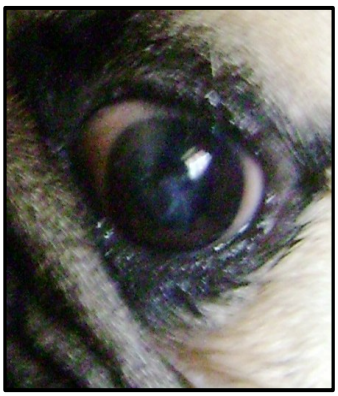

Fig. 16. Cleared cornea with central haziness on 60th day (Dog No. X)

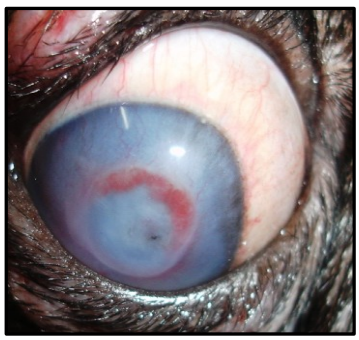

Fig. 18. Corneal vascularization on 14th day (Dog No. V)

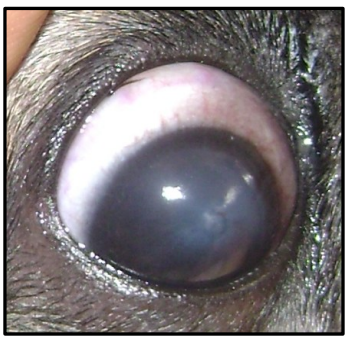

Fig. 19. Complete clearing of cornea on 60h day (Dog No. V)

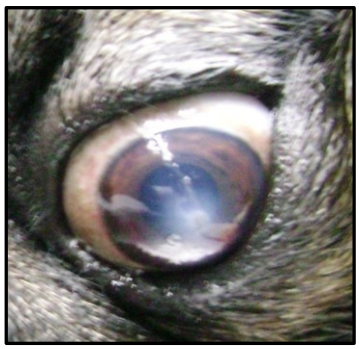

Fig. 20. Cleared cornea with central haziness on 45th day (Dog No. VI)

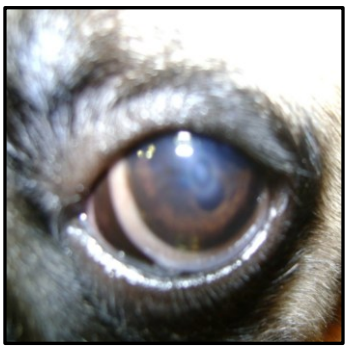

Fig. 21. Corneal haziness at center of the lesion on 45th day (Dog No. XII)

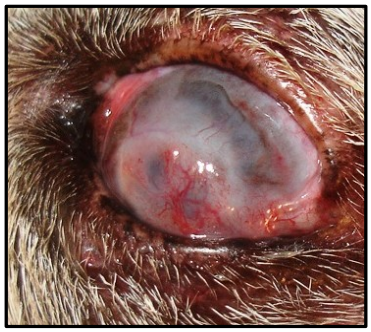

Fig. 22. Highly vascularized cornea on 28^{h} day (Dog No. XI)

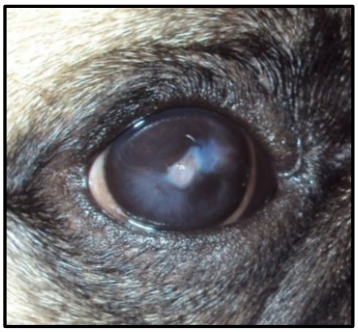

Fig. 23. Moderate opacity of central cornea and pigmentation on 60^{h} day (Dog No. IV)

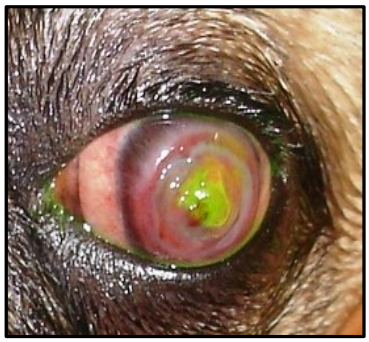

Fig. 24. Retention of fluorescein dye on 7th day (Dog No. VIII)

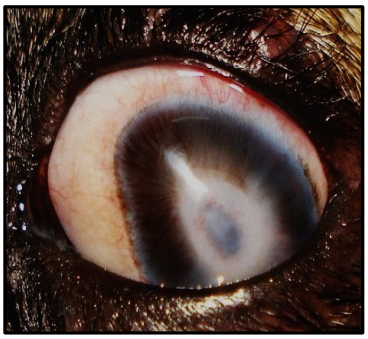

Fig.25. Corneal opacity on 60th day (Dog No. VII)

Discussion

5. DISCUSSION

5.1. SELECTION OF CASES

The study was carried out in twelve cases presented to the Veterinary hospitals at Kokkalai and Mannuthy and diagnosed to have corneal ulcers. Among these cases, six cases were included in Group I (Dog Nos. I, II, III, IV, V and VI) and were subjected to scarification of the lesion followed by application of collagen sheet. The other six cases were included in Group II (Dog Nos. VII, VIII, IX, X, XI and XII) and were subjected to scarification or superficial keratectomy. In them eight cases *viz.* Dog Nos. I, II, III, IV, VII, IX, XI and XII had staphyloma and four cases *viz.* Dog Nos. V, VI, VIII and X with deep stromal corneal ulcers.

Among twelve animals selected five were females and rest were males. Among them, both right (4) and left (8) eyes were involved. The average age of animals selected for the study was 13.58±5.06 months, ranging from one month to 5 years. Current study included five puppies, four young adults and rest were juveniles. This observation was not in accordance with Murphy *et al*. (2001), Bentley, (2005) and Janssens (2007), they reported the occurance of superficial chronic corneal erosion or indolent ulcers were more with middle aged to older dogs.

The exciting cause of injury was not obvious except in Dog No. IX due to inadequate history but involvement of trauma was suspected in almost all cases. In Dog No. IX the condition was subsequent to road accident. There were no specific reports regarding the occurrence of corneal ulcers in dogs of different sex. The higher incidence of corneal trauma in young ones may be due to the restless behavior and playfulness.

Out of twelve dogs selected for the study all except one were Chinese Pugs. This observation is in accordance with Startup (1984), Wolfer and Grahn (1994), Whitley *et.al.* (1995) and Raji (2006) who reported a higher incidence of corneal lesions in brachycephlic breeds including pugs. The presence of shallow orbits, excessive prominence of the globe, decreased corneal sensitivity, and reduced tear film stability (Hendrix and Cox, 2008) and the inherited corneal insufficiency, poor corneal reflex and lack of protective eye consciousness (Startup, 1984) were suggested as reasons for high incidence of corneal lesions.

5.1.1. Signalment and Anamnesis

The average age of dogs under this study was 13.58 ± 5.06 months ranging from one month to five years. Chinese Pugs were overrepresented in this study. Out of the twelve cases, eleven were Chinese Pugs, and one was Mongrel dog. This was supported by the findings of Startup (1984) that the breeds with large corneal surfaces, lowered surface temperature and sensitivity of the cornea, inherited nerve deficiencies are predisposed to the corneal ulcers. Wolfer and Grahn (1994) and Hendrix and Cox (2008) pointed out that brchycephalic breeds are predisposed to corneal trauma due to lagophthalmos, globe prominence and decreased corneal sensitivity.

The duration of illness of the cases under this study ranged from two days to three weeks. Most of the cases were complication of simple corneal ulcer, which might have resulted from trauma to the eye by the companion animal or by self mutilation.

In most of the cases staphyloma and adhesion of iris to the corneal wound was developed. Corneal pigmentation was already developed which might be due to the constant irritation in majority of the cases. Corneal ulcers persisted for a long period was considered as refractory ulcers. Re-epithelialisation of uncomplicated ulcers needed three to five days, but if they do not heal within this

period could be considered as refractory ulcers (Whitley, 2000 and Mandell and Holt, 2005).

5.2. SURGICAL TREATMENT

All the cases in Group I and II were subjected to surgical treatment and were carried out under general anaesthesia with proper preoperative preparation.

5.2.1. Preoperative Preparation

All the cases were put under medical therapy with topical application of ciprofloxacin eye drops four times daily for three days prior to surgery in Dog No. III, V, VI, VIII, X, XI and XII and were found sufficient to control infection. The corneal penetration of drugs were markedly increased when there was corneal epithelial cell loss (Munro, 2001).The topical broad spectrum antibiotic should be administered six to 24 hours prior to surgery especially in infectious keratitis and corneal perforation (Wilkie and Whittaker, 1997).

Fluoroquinolones have greater efficacy and broader spectrum of activity, and found effective against bacterial keratitis with a variety of pathogens (Kern, 2004). Hendrix and Cox (2008) found out that topical application of ciprofloxacin resulted in a mean tear concentration of ciprofloxacin that remained above the MIC_{90} levels for most pathogenic bacteria for 6 hours in normal mesocephalic and brachycephalic dogs.

All the dogs were fasted for 12 hrs before surgery and withheld liquid food for four hours prior to surgery. The adenexal areas were scrubbed with povidone-iodine solution before surgery. The eye was irrigated with normal saline and a few drops of povidone-iodine (1: 50) solution were instilled before surgery.

Povidone-iodine solution and sterile saline solution were effective in eliminating the bacterial contamination of the lid margins and dilute povidone – iodine solutions produce less interference with the normal host tissue health and defenses (Roberts *et al.* 1986 and Morgan 2004). Dilute (1:10 to 1:50) povidone-iodine is an effective topical antiseptic for veterinary ophthalmic surgical preparations and can be safely applied to the eyelids and corneoconjunctival surface (Herring, 2003).

5.2.2. Anaesthesia

In this study, anaesthetic regimen including atropine sulphate, xylazine hydrochloride, ketamine hydrochloride and diazepam provided sufficient anaesthesia for surgeries. Instillation of 4% lignocaine hydrochloride produced topical anaesthesia satisfactory for eye examination and for measuring IOP. Analgesia was found to improve the animal's comfort and minimize the tendency for self mutilation because of the rich innervations of the cornea.

The potential benefit of anticholinergics was prevention or reversal of oculocardiac reflex. In ketamine administration, the palpebrae remained open and globe was centrally positioned and the corneal and palpebral reflexes persisted. Diazepam reduced the ketamine induced increased IOP. Analgesia was found improving animals comfort and minimized tendency to self trauma. Topically administered anaesthetic may be irritating and can cause transient conjunctival hyperemia. It can be damaging to corneal epithelium and delay corneal wound healing when used continuously (Collins *et al*. 1995) which was absent in any of the cases.

5.2.3. Surgical Technique

5.2.3.1. Corneal debridement

Debridement was carried out in all the cases in this study. Cleanliness of cornea and conjunctival sac is essential in the treatment of corneal ulcers and is best accomplished with the use of saline irrigations. Saline irrigations were used in order to remove the dead and necrotized tissues in the eye. Necrotic tissues and the fibrin clots over the corneal ulcer often require debridement or scarification. All the loose corneal epithelium from the area of corneal ulcers and any surrounding cornea were removed using a sterile cotton swab in a circular, rubbing motion. The debridement technique alone offered an acceptable rate of healing, but resulted in a longer recuperative period (Stanley *et al.*, 1998). Debridement stimulated corneal healing by the proliferation of adjacent epithelium and promoted the attachment of basement membrane (Moore, 2003).

5.2.3.2. Superficial keratectomy

Superficial keratectomy was carried out in all the cases and removed the necrotized and redundant corneal portion. Superficial keratectomy was indicated in infectious keratitis as reported by Wilkie and Whittaker (1997) and Moore (2003), and also in neoplastic and non neoplastic conditions, excision of necrotic material and in retained corneal foreign bodies (Herring, 2003). Here the redundant epithelium, the abnormal basement membrane, and part of the underlying corneal stroma were removed to allow for the migration of normal epithelium and deposition of normal basement membrane (Pickett, 1995).

Stanley *et al*. (1998) and Moore (2003) reported that this procedure removed the acellular zone of hyaline collagen in the anterior stroma thus eliminated the barrier to epithelial healing and strengthened the adhesions of epithelium to the corneal stromal collagen. It exposed the migrating corneal

epithelial cells to the sub epithelial type I collagen and resulted effective attachment between the migrating epithelium and stroma. The main complications of superficial keratectomy included infection, granulation, pigmentation, perforation and scar formation especially in brachycephlaic breeds (Stanley *et al*., 1998).

5.2.3.3. Application of collagen sheet

Potential biomaterials for the repair of corneal defects include collagen, collagen-hydrogel polymers, bioactive synthetics and coated hydrogels and small intestinal submucosa which acted as collagen based biomaterial. As a biomaterial graft for corneal repair, small intestinal submucosa offered advantages in being inexpensive, readily obtainable and technically straightforward to place surgically (Feathestone *et al*., 2001 and Hollingsworth, 2003).

The intestinal submucosal collagen sheet of bovine origin in desired size and shape was thoroughly washed with sterile normal saline and was moistened with gentamicin eye drops was kept over the cornea in all the cases under Group I. There were no adverse reactions in any of the dogs under this study. The collagen sheet was completely absorbed by the third post operative day and the healing progression was found to be drastic in all the cases. Complete reconstruction of the cornea was observed in severely damaged cases of staphyloma (Dog Nos. I, II, III and IV).

Geasey *et al*. (1992) reported that collagen shields were proved to be highly oxygen permeable, promote epithelial healing, decrease inflammatory cell infiltration and reduce stromal oedema. According to Bussieres et al. (2004) small intestinal submucosa acted as a scaffold for the repair and provided valuable tectonic support and epithelization of small intestinal submucosa occured because of its collagenous nature mimics the stromal surface.

Raji (2006) used collagen graft made from bovine Achilles tendon for management corneal lesions and obtained negative fluorescent dye test by about 7 days and achieved almost complete clarity by about 90 days.

The small intestinal submucosa graft may be an effective alternative surgical treatment to the traditional conjunctival grafts commonly used to repair melting ulcers in dogs and cats. The advantages of using a small intestinal submucosa graft included good corneal transparency, preservation of corneal integrity and maintenance of vision (Vanor *et al*., 2007).

5.2.3.4. Temporary tarsorrhaphy.

Temporary tarsorrhaphy was employed in all the dogs in this study. It is placed as a protective bandaging technique and useful as a part of treatment for corneal ulcers also (Moore, 2003). It allowed the postoperative application of topical medication through the medial canthus and improved postoperative healing (Herring, 2003). Partial disruption of tarsorrhaphy sutures were observed in Case No. III and VIII.

5.2.4. Post Operative Care

Parentral administration of cephalexin at the rate of 20 mg/kg body weight and topical applications of ciprofloxacin and flurbiprofen eye drops effectively controlled infection, inflammation and post operative pain in all animals except Dog No. X where topical application of chloramphenicol was done based on the culture and sensitivity result. Antibiotics were indicated in the treatment of corneal ulceration as the loss of epithelial barrier exposes the corneal stroma, which can become colonized by bacteria (Munro, 2001). According to Sansom (1988), an established intraocular infection was difficult to treat due to the presence of blood ocular barrier and absence of lymphatics. So in case of infection both systemic and topical treatments should be considered.

Flurbiprofen controlled the post operative inflammation in the present study (Sansom, 2000). This is in agreement with the observation made by Munro, 2000 and Giuliano, 2004 that topical non-steroidal anti-inflammatory agents were generally recommended to reduce post operative inflammation.

Elevated intraocular pressure in Dog Nos. I, II, V, VI, VII, VIII, IX and X were managed successfully with oral administration of acetazolamide at the rate of 20 mg/kg body weight and topical administration of dorzolamide in Dog No. II and timolol maleate in Dog No. V, VII and IX. Topical administration of timolol maleate resulted in a reduction of IOP in treated and contralateral eyes of normotensive dogs and cats and peak effect was seen between two and four hours after the treatment in dogs (Willis, 2004).

In the present study Elizabethan collar was found useful in preventing self mutilation and was well tolerated by the animals in the postoperative periods which agrees with the observations of Startup, 1984; Collins et al., 1995; Moore and Constantinescu, 1997 and Herring, 2003.

5.3. MAIN ITEMS OF OBSERVATION

5.3.1. Physiological Parameters

None of the animals showed any obvious sign of systemic illness as evidenced by the examination findings. Because the ocular involvement may indicate systemic diseases, a general physical examination should preceed the ophthalmic examination as per Felchle and Urbanz (2001). There was no significant variations in the physiological parameters in both groups. All the animals were devoid of any systemic infection and surgical manipulation was done in all the cases.

5.3.2. Clinical Examination

5.3.2.1. General condition of patient

All the animals were in good body condition except Dog No. I, which had poor condition on the first day and recovered good health in later observations. Multivitamins were provided in all the cases. The glycogen on the corneal epithelium act as the main source of energy under stressful conditions like trauma or surgical wounds and therefore, if the glycogen stores got depleted, normal healing of epithelium and cellular locomotion over the surface would be inhibited (Gum, 1991).

5.3.2.2. Wet film examination

Moving blood parasites were not observed preoperatively in any of the dogs under the study.

5.3.2.3. Condition of eye

5.3.2.3.1. Nature of discharge

Nature of the discharge was purulent in all dogs under Group I except Dog Nos. V and VI on the day of presentation. It became clear by the 7^{th} day of observation. Except Dog No. X, all the cases in Group II were presented with pustular/ purulent discharge that became clear on 14^{th} day of observation. In all the cases the discharge was clear in the later observation period. The purulent discharge usually indicated a bacterial infection.

5.3.2.3.2. Type and extend of lesion

In Group I, Dog Nos. I and II were presented with complete damage of cornea and with prolapse of the iris resulting in the collapse of anterior chamber. Staphyloma required immediate repair as it will lead to intraocular infection (Bedford, 1987). In Case Nos. III and IV corneal perforation occurred and complicated by the formation of staphyloma.

Dog Nos. V and VI were presented with deep stromal corneal ulceration. Corneal ulcers may turn to deep stromal ulcers as a result of infection, eyelid abnormalities and use of corticosteroids (Wilkie and Whittaker, 1997). Deep and progressive ulcers were vision threatening (Whitley, 2000) and required corneal vascularization and took about three weeks to heal (Moore, 2003 and Mandell and Holt, 2005).

The collagen sheet applied over the cornea after scarification of ulcer in all the cases in group I were completely dissolved by 3rd day. In all the cases, the depth and extend of the lesion got reduced and become shallow on 3rd day itself. The corneal lesions in all cases were completely covered by 28th day of observation and regained the corneal surface. The collagen sheet may act as a substrate for the stroma and reconstruct the cornea.

In Group II, cornea of Dog Nos. VII, IX and XII were ruptured and resulted in staphyloma on the day of presentation. In Dog Nos. VII and IX were more complicated and anterior chamber was collapsed. The size and depth of the lesion in Dog Nos. X and XII were reduced progressively and covered by 45th day of observation. In Dog No. XII the cornea became clear and the corneal haziness persisted in Dog No.X by 60th day.

In Dog No. VIII cornea was completely damaged but did not penetrate in full depth and stromal ulceration was stationary in nature. The lesion was covered by the 45th day of observation. Cornea was completely damaged and infected in Dog No. XI and the iris was covered by the exudates and fibrin clots. Corneal healing was observed by 14th day and regained its shape by 28th day. By 45th day cornea was reconstituted but clarity was completely absent.

Apart from superficial keratectomy, conjunctival flaps and corneal grafting, hydrophilic contact lenses were suggested for the treatment of corneal ulcer and staphyloma (Startup, 1984; Wilkie and Whittaker, 1997 and Herring, 2003).

5.3.2.3.3. Visual function evaluation

All the visual function tests could not be conducted in all dogs. Visual reflexes could not be assessed due to severity of corneal damage and also due to corneal oedema and opacity. As per Felchle and Urbanz (2001), visual function tests like menace reflex and pupillary light reflex test were used. The papillary light reflex test evaluated the function of retina, optic nerve and the iris sphincter muscle. Pupillary reflexes can be evaluated only when the cornea is clear.

5.3.2.3.4. Corneal clarity

On the day of presentation, all the dogs were presented with the loss of corneal clarity. According to Morreale (2003), factors essential to maintain corneal clarity included lack of blood vessels or pigmentation, the size and regular arrangement of the collagen fibrils of the stroma and relatively dehydrated nature of cornea. Featherstone *et al*. (2001) reported that during normal stromal healing, type 3 collagen was laid down and was less regularly arranged, thus resulted in corneal scarring and possible visual impairment.

Clarity of cornea was restored only in dogs presented with stromal ulcers and the clarity was lost in severely damaged corneas with staphyloma, where the cornea was newly formed. This observation was supported by the findings of Wilkie and Whittaker, (1997) that opacification of cornea was frequently observed with healing of a wound which may be irreversible due to the deposition of a type of collagen fibrils that are not characteristic of cornea. Disruption of the regular lamellar arrangement of the stroma or change in collagen type leads to the development of opacity.

Since the observation was made for a maximum period of 60 days complete clarity of the cornea could not be observed in all cases. Clearing requires more than three months. However clarity was achieved in uncomplicated cases within 60 days.

5.3.2.3.5. Corneal oedema

Corneal oedema was observed in all the dogs under this study and a progressive reduction was there in all cases during the subsequent observation periods. Corneal opacity was found as a common symptom of keratitis (Wilcock, 1993 and Wilkie and whittaker, 1997). Corneal fluid is hypotonic to both tears and aqueous humor and any break in either epithelium or endothelium increased the fluid content and lead to corneal oedema (Startup, 1984). Alteration in the endothelial cells resulted in the cornea absorbing aqueous humor and became oedematous (Gilger *et al*., 2008).

5.3.2.3.6. Vascularization of cornea

Vascularization of cornea was observed in all the cases under this study. In Group I, it was found to be increased in intensity and reached the peak by 7^{th} day and persisted till 14^{th} day of observation. There after it decreased. Corneal

vascularization is an important part of corneal healing (Startup, 1984 and Mandell and Holt, 2005). The corneal neovascularization is almost similar in Group II also. After the completion of the epithelization topical corticosteroids were applied inorder to remove the vascularization and concurrent pigmentation.

Corneal vascularization represented an emergency reaction to improve the nutrition of the cells of the cornea in different pathological processes and to support the process of healing (Magrane, 1977).

Neovascularization of cornea following the implantation of small intestinal submucosa is probably due to the surgery, initial traumatic event, and to corneal repair rather than an immune rejection (Bussiers *et al*, 2004).

But uncomplicated stromal wounds healed without vascularization and the stromal vascularization was depending upon the duration of ulcer and developed after three to five days (Wilkie and Whittaker, 1997 and Slatter and Dietrich, 2003). In all the cases blood vessels were completely withdrawn after the cornea was healed (Brunott *et al*., 2007).

5.3.2.3.7. Fluorescein dye test

Fluorescein dye strips were used for assessing the depth, extent and healing of corneal lesions. Fluorescein dye test was positive in all the cases on the first day of observarion. The hydrophilic nature of the corneal stroma was responsible for the retention of the water soluble sodium fluorescein dye (Wilkie and Whittaker, 1997 and Ollivier, 2003).

In Group I, all the cases except Dog No. IV the test became negative on 7^{th} postoperative day which demonstrated the presence of an intact corneal epithelium. In Dog No. IV dye retention was observed on the centre of the lesion on 7^{th} day. The water soluble fluorescein does not penetrate the intact lipophilic

corneal epithelium, but do stain the intercellular spaces of the corneal stroma when the corneal defects were present (Helper, 1989 and Gelatt, 1991).

In Group II, fluorecein dye retention was observed till 14th post operative day in all the cases except Dog No. X, where it was observed on 7th day. The test remained positive till 28th day in Dog No. IX and thereafter it became negative. Thus the complete epithelization occurred after 28th day only.

These observations pointed out that the complete epithelization occurred early in Group I where collagen sheets were used. Collagen sheet was acting as a scaffold for the epithelization and resulted in a faster healing in Group I. It was in accordance with Gelatt and Gelatt (1994), they stated that irrespective of the nature of the graft structure, the structure of the recipient bed has to be simulated in the graft as it acts as a scaffold for the host corneal cells to migrate, as a source of corneal stroma and collagen lamellae and not as a source of viable cells.

5.3.2.3.8. Schirmer's tear test

Schirmer tear test results were within the normal range in all the cases except Dog No. II, which had a reduced tear production on the day of presentation. The application of artificial tear was advised in it and tear production became within normal range in all later observations.

Morreale (2003) described Schirmer's tear test as the most common test for pre corneal tear film and it asses the quantitive production of the aqueous portion of the tear film, which commonly performed before topical anaesthesia. The normal Schirmer's tear test in a dog ranged from 14 to 25 mm per minute. A Schirmer tear test is indicated in corneal ulcer because a normal tear film is required to maintain an intact corneal epithelium (Miller, 2001).

5.3.2.3.9. Other relevant observations

In Group I, increased intraocular pressure was observed in Dog Nos. I, II, V, VI, and Dog No. VII, VIII, IX and X in Group II. Beneficial reduction in the intraocular pressure was obtained by the treatment with acetazolamide tablets (Dog No. I, V, VI, VII, VIII, IX and X) and dorzolamide eyedrops (Dog No. II). In Dog No. V acetazolamide tablets were stopped after one week because of the respiratory distress and timolol maleate eyedrops were advised. In Dog No. VIII and IX in addition to it, timolol maleate eyedrops were also applied (Willis, 2004).

Pigmentation of cornea was observed in all the cases except Dog No. V, VI and XII. Pigmentation started at the corneal side of the limbus and coalesced and later interfered with the vision. Migration of the pigment through the nerves and vessels to cornea in staphyloma and anterior synechia was reported by Magrane (1977). Epithelial pigmentation is more common in chronic corneal diseases and stromal pigmentation was associated with severe corneal diseases (Slatter and Dietrich, 2003).

5.3.3. Haematological Parameters

All the haematological parameters were within the normal range and did not reveal significant difference between Group I and Group II. This was in accordance with the findings of Schalm *et al*. (2000); Raji, 2006 and Resmi, 2008. Serum blood glucose levels were within the normal range in all the animals.

5.3.4. Culture and sensitivity test

The culture and sensitivity of corneal swabs carried out in ulcerative keratitis helped in definitive diagnosis and selecting appropriate antimicrobial therapy (Wilkie and Whittaker, 1997).The material was collected before the application of any medication as it may inhibit the bacterial growth and tested according to the method adopted by Miller and Crenshaw (1988) and Massa *et al.* (1999).

Gram positive cocci were isolated in Dog No. I, II, IV, V and X Gram positive coccobacilli was isolated in Dog No. VIII. Gram positive cocci and Gram negative bacilli were concurrently isolated in Dog No. XI. So the most predominant bacterial isolate was Gram-positive cocci (46.2%), followed by Gram negative bacilli (7.7%) and Gram positive coccobacilli (7.7%). Gram positive organisms are most commonly isolated in the normal flora of canine eye and the pathogenic organisms involved in bacterial corneal diseases are usually the part of the ubiquitous and indigenous microflora of corneal and conjunctival surface, and invases when the corneal defence mechanism is compromised (Ollivier, 2003).

The most common bacterial isolates from infected ulcers were *Pseudomonas* spp. and *Staphylococcus* aureus (Morreale, 2003). Endogenous *Staphylococcus* spp. (gram positive cocci) are the most commonly isolated organism from conjunctival and eyelid swabs and are considered as the potential pathogens (Hamor, 2001).

In Dog Nos. III, VI, VII, IX and XII no growth was obtained after incubation for 24 hrs. It may be because of the current use of the antibiotic eye drops in them and in accordance with Massa *et al.* (1999) who opined that the use

of antimicrobials may interfere with culture and sensitivity test and do so with diagnosis.

The antibiotic therapy was selected based on the culture and sensitivity result and this was found to be a very reliable method in all cases of corneal injury, ulceration or perforation.

Summary

Summary

The efficacy of collagen sheets for the healing of corneal ulcers was studied and compared it with the healing of corneal ulcers without the use of collagen sheet in dogs. Dogs presented with the history of corneal affections were thoroughly examined and twelve dogs were randomly selected and divided into Group I and Group II each with six cases for the study. The clinical conditions included two stromal corneal ulcers and four staphyloma in each group. The average age of animals were 13.58 ± 5.06 months ranging from one month to 5 years and belonged to various breeds like Chinese Pug (11) and Mongrel (1) of either sex (seven males and five females).

All dogs selected for the study were examined for their general condition, haematological parameters, physiological parameters, nature of the discharge, type and extent of lesion, corneal clarity, corneal oedema, vascularization, fluorescein dye retention, and tear production. Wet film examination for the presence of moving blood parasites was conducted. Culture and sensitivity studies of corneal swabs were carried out on all cases to select the suitable antibiotic therapy.

All dogs were put under medical therapy with topical application of ciprofloxacin eye drops prior to surgery and fasted them for 12 hrs before surgery. The eye and adnexa were prepared aseptically for the surgery.

All the cases in Group I and II were subjected to surgical treatment and were carried out under general anaesthesia with proper preoperative preparation. Intramuscular injection of atropine sulphate 0.045mg/kg body weight and xylazine hydrochloride 1.5mg/kg body weight for premedication and ketamine hydrochloride 5mg/kg body weight for induction was used. A mixture of equal volumes of xylazine and ketamine (average 1.5ml) along with diazepam (0.5mg/kg body weight) were given intravenously ‘to effect’ for the maintenance

of general anaesthesia. The anaesthetic procedure was found satisfactory for surgical procedures in all dogs.

In all the dogs surgical procedures like scarification and/or superficial keratectomy was performed after the correction of staphyloma in order to remove the damaged tissue and to stimulate the healing process. In Group I, antibiotic soaked collagen sheet of bovine intestinal origin in desired shape was applied over the corneal bed and was sufficient to cover the lesion. Whereas in Group II, only scarification and / or superficial keratectomy was done. Temporary tarsorrhaphy was done in all dogs as a protective bandage and were removed on seventh postoperative day.

Elizabethan collar was applied in all cases in order to prevent self mutilation of the eye and was well tolerated by all the dogs. Oral medication with cephalexin and topical administration of ciprofloxacin and flurbiprofen was effective in controlling postoperative infection and inflammation. In one dog ciprofloxacin was changed to chloramphenicol based on the culture and sensitivity result.

Oral administration with acetazolamide was given in eight dogs with elevated intraocular pressure. Topical administration of timolol maleate in three cases and dorzolamide in one case was applied in addition for effective control of increase in intraocular pressure.

Collagen sheet applied over the cornea was found dissolved completely by third day in all the cases and no remnants were appreciable on the corneal surface. No adverse reaction to the material was noticed in any dogs and was well tolerated by them.

Physiological parameters like temperature, pulse and respiration were within the normal range in all the dogs and did not have any significance difference between the two groups throughout the observation period. All the dogs were in good body condition during the observation period and free of any moving blood parasites on wet film examination.

Nature of the discharge was purulent in all the dogs in Group I except two. But the discharge became clear by 7th day after the application of collagen sheet. In Group II except one case, all other cases were presented with purulent discharge and it resolved by 14th postoperative day.

In Group I, four dogs were presented as cases of complete corneal damage and staphyloma. Other two cases were of deep stromal ulcers. All the dogs were responded well to the application of collagen sheet after scarification and keratectomy. In all the dogs, the depth and extend of the lesion got reduced and become shallow on 7th day itself. The corneal lesions in all the cases were completely covered by 28th day of observation and regained the corneal surface. But the central corneal opacity was persisted in all cases except in two dogs'. Group II also consisted of four cases of staphyloma and two deep stromal ulcers. All of them were well responded to scarification and/or superficial keratectomy, but corneal healing took prolonged time when compared with Group I.

Corneal clarity was regained only in dogs with stromal ulcers in group I and corneal opacity in the centre of the lesion was observed in three dogs and haziness in one dog. In Group II, clearing of cornea was obtained in one dog but remained hazy in one and moderate opacity in was observed in two dogs. Corneal clarity was lost in rest of the dogs. Corneal pigmentation was observed in most of the cases except three and vision was compromised in them.

Corneal oedema was observed till 28th postoperative day in all the dogs in Group I except two, where it resolved after 14th day. In Group II, corneal oedema

was observed up to 14th day in one dog, 28th day in two dogs and 45th day in another two dogs. In one dog oedema was remained throughout the observation period.

Vascularization of the cornea was observed in all the dogs in Group I and it progressively increased in 7th day and remained till 14th day except in one dog where it resolved by 14th day. Thereafter vascularization was progressively regressed in all dogs. In Group II, vascularization of the cornea was observed in 28th day in three cases and thereafter it resolved progressively. In rest of the cases corneal neovasccularzation was resolved after 14th observation day.

In Group I, fluorescein dye test was negative by 3rd day in three dogs and by 5th day in one dog and by 7th day in one dog after the application of collagen sheet. Positive fluorescein test was observed only in one dog on 7th postoperative day and became negative thereafter. Thus complete reepithelization of the ulcer was observed by 7th post operative day in all except one dog. Whereas in Group II fluorescein dye retention was observed in all dogs up to 14th post operative day except one, where epithelization was completed within 14th postoperative day. Dye retention was seen up to 28th day in one case.

Tear production in all dogs under this study was within the normal range of 15 to 25 mm per minute.

Haematological parameters were found within the normal range in both groups throughout the observation period.

Ciprofloxacin was primarily selected as the antibiotic for topical application in all the dogs. The cultures obtained from the corneal swabs were sensitive to ciprofloxacin in three dogs and to enrofloxacin in three dogs. Since they belongs to the same quinolone group, ciprofloxacin was not changed. One

culture was resistant to ciprofloxacin but sensitive to chloramphenicol where the postoperative treatment was changed.

Topical antibiotic eye drops and anti-inflammatory agents were advised in selected cases, where an infection, pain and inflammation were noticed.

From the present study following conclusions were made

- Brachycephalic breeds like Chinese Pugs were more susceptible to corneal ulcers.
- Incidences of corneal lesions were more common in puppies.
- Broad spectrum antibiotics like quinolones were found effective in controlling the infection and can be chosen as the drug of choice before getting the results of culture and sensitivity test.
- Corneal culture and sensitivity were effective in selecting the appropriate antibiotic therapy.
- Fluorescein dye test was highly effective as a diagnostic tool and aid in assessing the depth and extend of corneal ulcers and also the progression of corneal healing.
- Complete dissolution of the collagen sheets were observed within three days and promoted the reconstruction of cornea without much delay.
- Temporary tarsorrhaphy was enough to retain the collagen sheet in position.
- Complete healing of corneal epithelium was observed within seven days after application of collagen sheet, which was demonstrated by fluorescein dye test.

- Complete corneal epithelization took about 14 days without collagen sheet.
- Pigmentation of the cornea was the major problem among the Pugs during the healing period which was difficult to heal.
- Anterior synechia and loss of corneal clarity were the important complications of corneal perforations.
- Collagen sheet was an acceptable biomaterial for the corneal lesions and was well tolerated by the dogs.
- Collagen sheet were found effective in enhancing the healing process in corneal ulcers as the healing was achieved within seven days.
- Collagen sheet is a good alternative to regain the normal corneal surface in corneal ulcer and in staphyloma.

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EFFICACY OF COLLAGEN SHEET FOR THE MANAGEMENT OF CORNEAL ULCERS IN DOGS

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ABSTRACT

The efficacy of collagen sheet of bovine intestinal origin in the healing of corneal ulcers were studied in six dogs and were compared with the healing without the use of it in another six dogs. Dogs presented with corneal ulcers and/or with staphylomas were clinically examined and selected for the study.

Surgical manipulations were performed under general anaesthesia. In Group I, collagen sheet was placed after scarification and/or superficial keratectomy and in Group II, scarification and keratectomy was performed. Temporary tarsorrhaphy was done in all dogs.

Oral administration of cephalexin was maintained in all the cases. Ocular instillation of ciprofloxacin as primary antibiotic or based on the culture sensitivity test and flurbiprofen were administered till the complete healing.

Elevated intraocular pressure was controlled by acetazolamide orally and by timolol maleate or dorzolamide topically.Collagen sheets of intestinal origin were completely dissolved by 3rd day and no remnants were seen. It was well tolerated by the dogs and no immune reactions were noticed. Since the collagen sheet was very pliable after soaking with gentamicin eye drops, it could be applied over the cornea very easily without any air spaces and retained in position by temporary tarsorrhaphy.

Fluorescein dye test became negative by 7th day in most dogs treated with collagen sheet and complete epithelization of the corneal defects was occurred by the time. Whereas the fluorescein dye retention was positive till 14th day in most dogs in Group II. Corneal vascularization developed in all the cases were resolved by the end of the observation period.

Complete reconstruction of the cornea was seen early in staphyloma cases under the collagen sheet treatment in Group I. But the clarity of the cornea was unable to regain within the observation period of the study in most cases. In stromal ulcers, the clarity was achieved by 60^{th} day.

The presence of anterior synechia was responsible for the delayed corneal clearing most of the staphyloma cases. Corneal pigmentation was the major complication encountered in either modality of treatment under this study.