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Assessment of corneal clarity, corneal thickness, grading and isolation of bacterial organisms in canine pigmentary keratitis[#]

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Abstract

A total of 13 corneas affected with pigmentary keratitis were selected for the study. Corneal clarity was assessed by visual examination and direct ophthalmoscope. Central and limbal corneal thickness was recorded with an Ultrasound pachymeter (PachPen). Pigmentation grade and mean pigment density (MPD) were assessed in all corneas. Isolation of bacterial organisms was performed through a corneal swab from all the corneas affected with pigmentary keratitis. Out of the 13 corneal swabs, the major organism isolated was staphylococcus aureus. The mean value of pigmentation grade was 55.3 ± 5.84 and the mean pigment density was 2.3 ± 0.24.

Key words: *Corneal pigmentation, PachPen, pigmentation grade, mean pigment density*

Corneal pigmentation or melanosis is developed as a part of corneal wound healing response after corneal trauma and with chronic tear deficiency due to keratoconjunctivitis sicca. Canine corneal pigmentation also occurs as a result of chronic irritation to the ocular surface like entropion, distichiasis, trichiasis, ectopic cilia and lagophthalmos due to facial nerve paralysis

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(Labelle *et al*., 2013). It is a common condition in Chinese pugs due to shallow orbits causing globe to be prominent and exposed and palpebral fissure is wide due to overlong eyelids termed euryblepharon (Appelboam, 2016). Corneal thickness is a physical feature which is related to corneal health and endothelial function (Villavicencio *et al*., 2014). Corneal thickness is measured using an ophthalmic pachymeter which uses ultrasound technology to record the corneal thickness (Hoehn *et al*., 2018). The corneas with pigmentary keratitis are divided to 24 sectors for grading of pigmentation (Allgoewer and Hoecht, 2010). Normal resident flora over the canine ocular surface is required for the health of the corneal surface. Profuse use of antibiotics and steroid drops enable the growth of opportunistic bacteria (Anoop *et al*., 2015b). This present study was conducted to evaluate the clarity, corneal thickness, pigmentation grading and isolation of bacterial organism in corneas affected with canine pigmentary keratitis.

The dogs with ophthalmic complaints presented to Teaching Veterinary Clinical Complex, Mannuthy and University Veterinary Hospital, Kokkalai during the period from October 2022 to October 2023 were evaluated and 13 corneas from 13 dogs formed the subject of the study. All the dogs were subjected to detailed clinical and ophthalmic examination. Corneas were closely examined using direct ophthalmoscopy for corneal clarity. According to Aswathy *et al*. (2023) clarity of corneas were scored from 1-4 (4-Clear, 3-Hazy, 2-Moderately opaque, 1-Completely opaque). The central and limbal corneal thicknesses were measured with ultrasound ophthalmic pachymeter (Pachpen). All the eyes were desensitised with 0.5% proparacaine eye drops. Nine readings each from the central and limbal cornea were taken. The average reading was recorded from the PachPen (Fig 1). The pigmentation was graded the using method proposed by Allgoewer and Hoecht, (2010). The affected corneas were schematically divided to 24 sectors (Fig 2). The pigment density was graded from 0-3 (0-None, 1-Fundus visible, 2-Iris visible, 3-Cornea opaque). From this the mean pigment density (MPD) was calculated by adding all the pigmentation grades divided by total no of sectors (Anoop *et al*., 2015a). The samples for corneal antibiogram were collected using sterile eye swabs before instillation of medication.

Corneal pigmentation, also known as pigmentary keratitis was developed as result of response to various irritating stimuli. Out of the 13 dogs presented eight (61.5%) animals were Chinese pugs, two were (15.38%) Shihtzu, one (7.69%) was a Golden retriever, one (7.69%) was a Labrador retriever and one (7.69%) was a non-descript dog. The findings were in accordance with Labelle *et al*. (2013), Antonia *et al.* (2014), Sarangom *et al*. (2014), Anoop *et al*. (2016) and Costa *et al*. (2021). According to Anoop *et al*. (2015a), Chinese pugs were

Table 1. Grading of pigmentation

Corneas	Number of sectors affected	Grade of pigmentation	Mean pigment density
1	10	23	0.958
2	24	68	2.83
3	24	72	3
4	24	72	3
5	24	67	2.71
6	24	72	3
7	8	20	0.83
8	24	72	3
9	16	31	1.29
10	24	72	3
11	21	54	2.25
12	24	66	2.75
13	12	30	1.25

Fig. 1. Pachymeter

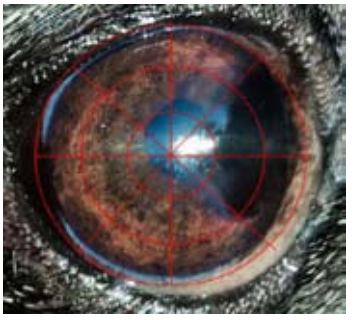

Fig. 2. Grading of pigmentation

Fig. 3. Staphylococcus in blood agar

prone to corneal pigmentation due to shallow orbit, excessive prominence of the globe, decreased corneal sensitivity, reduced tear film stability, poor corneal reflex, lack of protective eye consciousness and many other factors. Venugopal (2013) observed that the excessive prominence of the globe in Chinese pugs predisposed to xerophthalmia and exposure keratosis.

Clarity of the cornea ranged from one to four with a mean clarity of 1.53 ± 0.183. Out of the 13 corneas presented seven corneas (53.8%) were completely opaque (score-1), five corneas (38.4%) were moderately opaque and one cornea (7.69%) was hazy. The mean value of corneal clarity was in accordance with findings of Thajunnisa *et al*. (2020).

The value of extent of pigmentation ranged from 20 to 72 and mean pigment density ranged from 0.83 to 3. The mean value of extent of pigmentation was 55.3 ± 5.84. The value of mean pigment density was 2.3 ± 0.24. Out of the 13 corneas, eight (61.5%) corneas were completely pigmented (extending to all 24 sectors). The findings were in accordance with Azoulay (2014).

Accurate measurement of corneal thickness was critical in ophthalmology and vision science. Furthermore, it might enable the identification of early corneal alterations associated with endothelium toxicity in the research context (Alario and Pirie, 2014). According to Hoehn *et al*. (2018), ultrasonic pachymetry (USP) generates measurements by making contact with the cornea and using ultrasound technology. Corneal thickness was measured both from the centre and limbal cornea. Out of the 13 corneas, two corneas did not record central corneal thickness (CCT) and four corneas did not record limbal corneal thickness (LCT), which was due to the extensive pigmentation, high pigment density, neovascularisation and opaque nature of the cornea (Wongchaisuwat *et al*., 2018). The mean value of CCT was 690.5 ± 33.3 µm and the mean value of LCT was 793.1 ± 31.3 µm. The values were within the normal range.

Corneal swab was collected from all the corneas for antibiogram. Out of the 13 corneal swabs, five (38.4%) corneas (C1, C3, C4, C5 and C13) did not show any growth even after 48 hours of incubation. Two types of organisms were isolated from three (23.07%) corneas (Fig 3). The major organism isolated was *Staphylococcus aureus* (53.8%), followed by *Klebsiella* sp. (15.3%), *β haemolytic streptococci* (7.69%), *Enterococcus* sp. (7.69%), and *Pseudomonas* sp. (7.69%). The results were in accordance with Das *et al*. (2019), Hewitt *et al*. (2020) and Thajunnisa *et al*. (2020). The normal ocular surface contained microbial flora which would interfere with invading microbes by depriving them of nutrients and secreting substances having anti-microbial properties. Thus, long term usage of ocular anti-biotics and steroids would destroy the normal ocular flora (Das *et al*., 2019).

Summary

Corneal pigmentation is the major reason for reduced corneal clarity in many of the brachycephalic breeds. The grading of pigmentation by dividing the cornea to 24 sectors was found to be effective in evaluating the severity of pigmentary keratitis. The bacterial organisms isolated were the opportunistic

pathogens and they do not have direct relation in the development of pigmentary keratitis.

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Conflict of interest

The authors declare that they have no conflict of interest.

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