ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Assessment of grading and isolation of bacterial organisms in Canine Pigmentary keratitis.

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Manuscript Info

Manuscript History:

Received: 15 February 2015
Final Accepted: 25 March 2015
Published Online: April 2015

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Abstract

A total of eighty three corneas of fifty five dogs presented with pigmentary keratitis were evaluated by assessing the grading technique. Pigmentation grade, extent of pigmentation and mean pigment density were calculated in all the corneas. Isolation and identification of the organism from the corneal swabs was done in all the corneas presented with pigmentation.The method of grading of pigmentation was found to be ideal for assessing the efficacy of treatment for pigmentary keratitis and the main organism isolated from the corneal swab was *staphylococcus aureus*.

INTRODUCTION

Pigmentary keratitis or Corneal pigmentation is a non-specific response of cornea to insult, associated with pigment proliferation, migration of melanocytes and neovascularisation of cornea. The location, extent and depth of the pigment deposition depend on initiating corneal insult (Crispin, 2005). It is a common condition in Chineese pugs and other brachycephalic breeds due to chronic exposure called euryblepharon causing a chronic corneal irritation (Goulle, 2012). The grading and assessment of corneal pigmentation before and after treatment poses a problem to the attending ophthalmologist. The resident flora of the canine cornea is important for protection of eye against infections. When resident flora is inhibited by disease or long term application of antibiotic or corticosteroid, opportunistic pathogens could expand to develop the disease (Kecova*et al.* 2004). Various organisms has been isolated from the canine corneal surface.The study was undertaken to grade the pigmentation of cornea and to isolate and identify the common ocular pathogens associated with pigmentary keratitis in dogs.

Materials and Methods.

The dogs with ophthalmological complaints specific to cornea presented to University Veterinary Hospital, Mannuthy for the period from January 2011 to June 2014, were evaluated for corneal pigmentation and 83 corneas from 55 animals were selected for the study. All the corneas were subjected to detailed clinical and ophthalmological examination. The corneas with pigmentation were graded as per the technique of Allgoewer and Hoecht (2010) and pigmentation grade, extent of pigmentation and mean pigment density were calculated in all the corneas. For this, the cornea was schematically divided in to 24 sectors (Fig.1). The division of cornea in to sectors compensated for the greater clinical importance for vision of the central vs. the peripheral sectors, by making the former smaller than the latter. Each corneal sector was evaluated for extent of corneal pigment and pigment density. Corneal pigment density was graded within the sectors from 0-3 (0-None, 1= fundus visible, 2= iris visible, 3=cornea opaque). Pigmentation grading of the particular cornea was calculated by adding all the sector grades (Allgoewer and Hoecht (2010). The maximum grading was 72 for a totally pigmented eye.For calculating **e**xtent of

pigmentation, the cornea was divided in to 24 sectors and the number of sectors affected with pigmentation was counted. The mean pigment density (MPD) was calculated by adding the products of the numbers of sectors and grade of pigmentation divided by total number of sectors.

The samples for microbial/ bacterial culture were collected by sterile swabs before instillation of any medication. The eyelids were gently retracted and the sides of the sterile swabs were rolled over the pigmented cornea without touching the eyelid margin or eyelashes. The samples were inoculated in Blood agar using quadrant-streaking method and the plates were incubated at 37°C for 24 hours for the presence of bacterial growth, if any.

Results and Discussion.

The grading of pigmentation of the cornea was done as per the method suggested by Allgoewer and Hoecht (2010). This method of calculation of pigmentation grading was supported by Denk*et al.* (2011) and Azoulay (2014). The mean value of pigmentation grading in 83 corneas was 32.59±2.27. Among 83 corneas only 5 corneas (6%) showed pigmentation grade of 72 (fully pigmented). The values of pigmentation grading ranged from 8 to 72. The mean value of extent of pigmentation was 15.67±0.83. Out of the 83 corneas, 26 corneas (31%) showed pigmentation extending in all the sectors. The values of extent of pigmentation ranged from 4 to 24. The mean value of mean pigment density was 1.37± 0.07. Out of the 83 corneas, 8(10%) corneas showed mean pigment density of 3. The values of mean pigment density ranged from 0.3 to 3.The mean pigment density (MPD) of a particularcornea was calculated by dividing the total pigmentation grade of the cornea with the total number of sectors. This is in agreement with the suggestions of Azoulay (2014). The maximum mean pigment density value was three and the maximum value of pigmentation grade was 72 (Fig.2).

The normal resident flora is an important part of eye's protection against infections. Opportunistic bacteria like *Staphylococcus epidemicus*, *E.Coli, Cornybacterium spp., Klebsiella* and *Bacillus w*ere considered either non pathogenic or to be weakly pathogenic. When resident flora was inhibited by disease or long term application of antibiotic or corticosteroid, opportunistic pathogens could expand and disease develops (Kecova*et al.* 2004) and Ollivier (2003). In the present study most of the animals were under treatment with an antibiotic eye drop for corneal ulceration or other corneal diseases. The main organism isolated from the corneal culture of dogs having varying grades of pigmentary keratitis was *Staphylococcusaureus*(Fig.3) in thirty (40%), followed by *Enterococci* sppin thirteen (16%), *Staphylococcus intermedius* in eight (10%), *Coranybacterium* spp. in ten (12%), *Klebsiella* spp. in six (7%), *Bacillus* spp. in three (4%), *E coli* in three (4%) and *Actinobactor* spp. in five (6%) samples. The swab from two corneas (2%) did not show any growth. The observation on the most common organism isolated from canine cornea as S*taphylococcus aureus* was corroborated by Massa *et al.* (1999), Whitley (2000), Cullen *et al.* (2005), Prado *et al.* (2005), Wang *et al*. (2008), Ramani*et al*. (2013) and Antonio (2014).

In majority of cultures from corneal swab a single type of organism was isolated and some cultures showed profuse and certain others showed only scanty growth of organisms. Ollivier, (2003) also reported that a single organism was isolated in 94% of dogs with external ocular disease, 2 organisms in 12 % and 3 or more organisms in 3% of dogs. The corneas with a heavy purulent discharge produced profuse growth of *Staphylococcus aureus.* The normal resident organisms or other opportunistic pathogens were not directly responsible for the development of corneal pigmentation but due to keratoconjunctivitissicca (KCS), there were reduction in normal resistance of corneal surface which favoured the organism to flare up. This is in agreement with the observation of Davidson and Kuonen (2004) that KCS might predispose ocular surface to infection and dogs with KCS had heavier growth of organisms like coagulase positive *Staphylococcs spp*. and *β haemolytic Streptococci* .

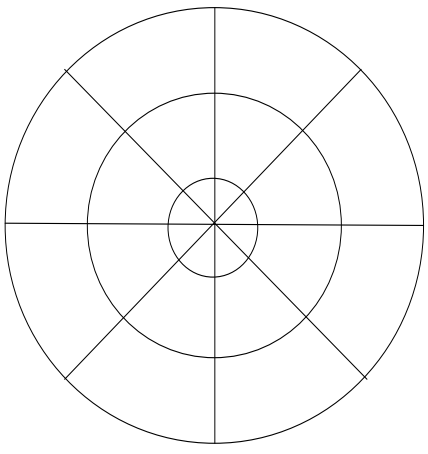

Fig.1. Schematic division of cornea in to 24 sectors for grading

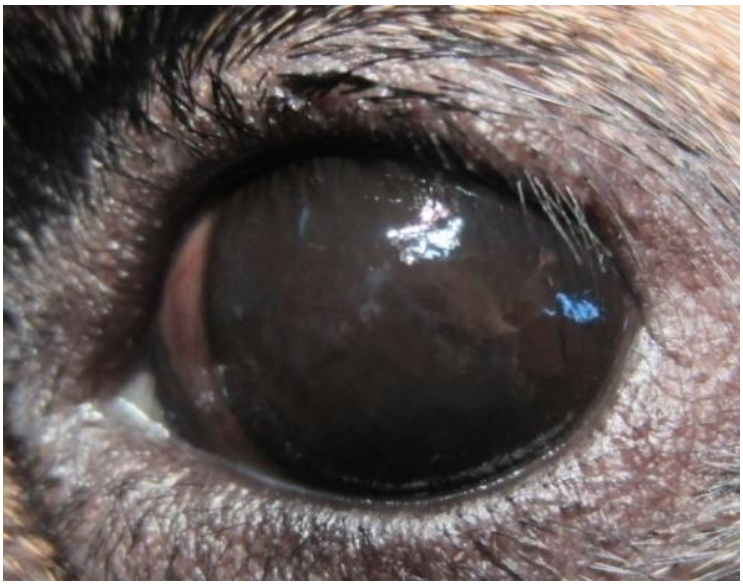

Fig.2. A cornea showing pigmentation grade- 72, extent of pigmentation- 24 and mean pigment density-3.

Fig. 3.*Staphylococcus aureus* in Blood agar

Summary.

The method of grading of corneas affected with Pigmentary keratitis by schematically dividing the cornea in to 24 sectors and assessment of Pigmentation grade, extent of pigmentation and mean pigment density was found suitable for evaluation and efficacy of treatment for corneal pigmentation. The bacterial organisms isolated from the cornea of dogs affected with Pigmentary keratitis represented the opportunistic pathogens and not directly related to cause of corneal pigmentation.

Acknowledgement

The authors are thankful to Kerala State Council for Science, Technology and Enviornment (KSCSTE) for providing financial assistance for carrying out this research work.

References.

1. Allgoewer, I. and Hoecht, S. 2010. *Vet. Ophthalmol.*13 (**1**): 20-25.
2. Antonia, N.A. 2014. MVSc thesis.Kerala Veterinary and Animal Sciences University.Pookode. 85p.
3. Azoulay,T. 2014. *Vet. Ophthalmol.* 17: 241-249.
4. Crispin, S.M.2005. *Notes on Veterinary Ophthalmology*. (1st Ed.) Black well, Iowa. Pp.215-230.
5. Cullen,C.L., Ihle,S.L., Webb,A.A and McCarville, C.2005. *Vet. Ophthalmol.* 8(4): 215-224.
6. Davidson,H.J and Kuonen, V.J.2004. *Vet. Ophthalmol*. 7(**2**): 71-72.
7. Denk,N., Fritsch, J and Reese,S. 2011.*Vet. Ophthalmol.* 14(3): 186-194.
8. Goulle, E. 2012. *J. Small Anim. Practice*. 53: 34-43..
9. Kecova,H., Hilinomazova,Z., Pauser,P and Necas,A. 2004. *Acta Vet.Brno.*73: 359-363.
10. Massa, K.L., Murphy, C.J., Hartmann, F.A., Miller, P.E., Korsower,C.S and Young,K.M.1999..*J. Am.Vet.Med. Asso.* 215: 1671-1674.
11. Ollivier, F.J (2003).*Clin.Tech. Small Anim. Pract*. 18: 193-198.
12. Prado, M.R., Rocha, M.F.G., Brito, E.H.S., Girao, M.D., Monterio, A.J., Teixeria,F.S and Sidrim,J.J.C. 2005. *Vet. Ophthalmol.* 8: 33-37
13. Ramani, C., Rambabu, K., D'Souza, N. J., Vairamuthu, S., Subapriya, S. and Justin William, B. 2013. *Indian J. Canine Pract*. **5(2):** 136-138
14. Wang, L., Pan, Q., Zhang, L., Xue, Q., Cui, J. and Qi, C. 2008.*Vet. Ophthalmol.* 11: 145-149
15. Whitley, R.D. 2000.*Vet.Clin. North Am. Small Anim. Pract.* 30: 1151-1165