Comparative efficacy of subconjunctival palpebral and bulbar injection of gentamicin-dexamethasone combination in dairy calves with epiphora

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SUMMARY

A prospective study was planned to compare the palpebral and bulbar subconjunctival injection of gentamicin-dexamethasone in dairy calves showing epiphora on exposure to dust pollution. Calves showing bilateral epiphora (n=12) and no epiphora (n=6) with mean age 6 months and weight 56.0 kg were included. The average periorbital matting of the facial hair extended to 4.8 cm. The value was 4.92 ± 0.59 cm and 5.17 ± 0.90 cm in the eyes subjected subsequently to epibulbar or subpalpebral injection respectively. Schirmer Tear Test values (mean ± s.e) in epiphoric eyes (27.67 ± 3.30 mm/min) were non-significantly higher than non-epiphoric (21.00 ± 0.97 mm/min). Conjunctival swabs revealed Gram positive and -negative commensals. One eye was injected 0.5 mL of the combination epibulbar and the contralateral subpalpebral routes. Calves in the control group received 0.5mL normal saline. In six calves, the epiphora subsided in three days and in four more it took seven days. Compared to day 0 values, the reduction of 98.9 ± 7.1% and 86.1 ± 10.9% respectively in facial matting did not differ significantly ($P > 0.05$). Recurrence or additional cases were not noticed subsequently in the farm. Moderate rains nine days following treatment of calves settled dust and reduced air pollution. It is concluded that the eyes in the calves are more prone than adults to the dust pollution. Both subpalpebral and epibulbar injection of gentamicin-dexamethasone are similarly effective in epiphoric calves. The studies evaluating the deleterious effects of air pollution on the ophthalmic health in food animals deserve more attention in future.

KEY WORDS

Calves; Epibulbar; Epiphora; Ophthalmology; Subconjunctival; Subpalpebral.

INTRODUCTION

Epiphora is defined as an abnormal flow of tears down the face[1]. Numerous etiologies that may lead to excessive tears include the following categories: appositional abnormalities of the eyelids, neurogenic lacrimal hypersecretory disorders, obstructive lacrimal drainage disorders, ocular surface disorders. The last two disorders are commonly encountered in bovine clinical practice. Unlike obstructive lacrimal drainage, the ocular surface disorders are predominantly encountered as outbreaks. Ocular surface dryness or irritation stimulates the reflex arc of the fifth and seventh cranial nerves, producing excessive tear secretion. When the lacrimal drainage system is unable to handle the increased tear volume, overflow occurs. Infectious Bovine Keratoconjunctivitis (IBK) or Pink Eye a severe transmissible ocular infectious disease caused by *Moraxella bovis* occurs during summer and autumn months when the fly populations are active[2]. The outbreaks of iritis and uveitis caused by *Listeria monocytogens* (silage eye) associated with silage feeding are also accompanied by epiphora[3]. However, in most of the animals with these disease conditions, the alterations in cornea, conjunctiva and or eyelids are easily noticed. Environmental factors and causes including stress, dust, UV light and foreign bodies[4,5,6]. Air pollution is a serious human health issue causing complaints of eye redness, irritation, watering, foreign body sensation, and blurring of vision. According to the World Health Organization (WHO), the air pollution consists of different particulate matter (PM) size. PM10 with particle aerodynamic diameter < 10 micrometer are generated from construction and the road dust[5].

Successful management of the tearing patient requires the clinician to determine the underlying cause of the epiphora. Being multifactorial in origin, the final diagnosis may always not be possible particularly under field clinical settings. Drugs may be delivered to the eye by topical application, subconjunctival injection, retrobulbar injection, intraocular injection and systemic administration. Systemic or topical use of antibiotics or a combination of both with or without corticosteroid are used in many infectious cases[3]. Subconjunctival administration of antimicrobials aims to reduce treatment costs and total dosages of drug while achieving higher ocular drug concentrations[7].

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Figure 1 - Dusty path leading to the grazing area.

The objective of this investigation was to compare the therapeutic efficacy of gentamicin-dexamethasone combination injected subconjunctival via palpebral or bulbar routes in calves with epiphora.

MATERIALS AND METHODS

The study was conducted in Mountain Livestock Research Institute (MLRI), SKUAST-Kashmir. Calves (n=34) were housed together in a room during the nights and in the paddock during the day time. They were fed concentrate feed 0.5 kg once and chapped fresh sorghum roughage 3.0 kg twice per day. Two sessions of grazing (2 hours each) in the nearby land were also allowed daily. The path leading to the grazing area and one located adjacent to the paddock was excessively dusty (Figure 1) due to prolonged dry spell.

The ailment (epiphora) developed naturally and suddenly in the calves included in this study. After obtaining consent from the head of the university farm, the treatment protocol was approved by the MLRI clinical board.

Fourteen[14] of the 34 calves suddenly showed bilateral (n=12) or unilateral (n=2) epiphora (Figure 2) in the second week of November 2020. Majority of the affected calves were Jersey (n=11), the remaining (n=3) crossbred Holstein Frisian.

Females (n=10) outnumbered the males (n=4). The mean age and body weight of the calves was 6 months and 56.0 kgs respectively.

The animals with epiphora were watched in the paddock one by one up to 10 minutes to identify any vision and ophthalmic anatomical defect, blepharospasm, photophobia, soiling or scratching the ophthalmic areas.

For detailed clinical examination, they were restrained in standing position by two attendants, one holding the head and other preventing backward or side way movement. Starting from the dependent inner canthus of the eye, the extent to which the overflowing tears had matted the facial hair was assessed using an ordinary scale (Figure 2). Palpebral and corneal reflexes were tested before holding the eyelids apart for close ophthalmic examination.

The Schirmer Tear Test (STT) was conducted in six calves (one eye per calf) with unilateral or bilateral epiphora. Six more calves (one eye per calf) with no epiphora (negative control) were also subjected to this test. The calves were restrained in standing with minimal eye manipulations. A STT strip placed in the lower conjunctival fornix was removed after one minute and the value recoded in millimeters.

Taking aseptic measures, conjunctival swabs were obtained from a total of 12 calves (one eye per calf); six animals with and six

Figure 2 - Calf with epiphora: matted facial hair assessment.

Figure 3 - Epibulbar injection in a calf with epiphora.

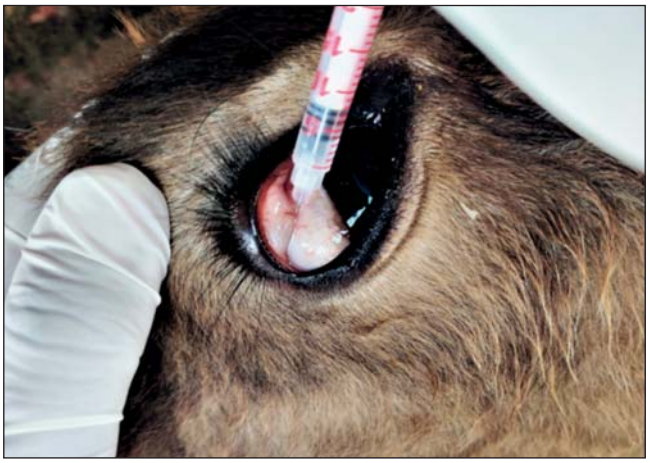

Figure 4 - Subpalpebral injection in a calf with epiphora.

more without epiphora. The swabs were inoculated on blood agar and maintained in aerobic incubation at 37 °C for 24 hours. All the calves including those of the control group were instilled homatropine hydrobromide eye drops (Homide, 2%, Indoco Remidies, Ltd) bilaterally and shifted to the adjacent room with low light for a period of 15 minutes. The head of the calves was subsequently restrained in standing position by an attendant for fundus examination using direct opthaolomscope (Heine Mini3000 LED, Germany).

The calves with bilateral epiphora (n=12) and those (n=6) without epiphora were then physically restrained in lateral recumbency on a table in the paddock and given subconjunctival injection. Using an insulin syringe, 0.25 mL gentamicin sulphate (Inj. Gentalab-40 mg/ml, Laborate Pharma India Ltd.) mixed with 0.25 mL dexamethasone sodium (Inj. Dexona- 4 mg/ml, Zydus Health care Ltd.) was injected per eye in all the calves (n=12) included in the treatment group. Those (n=6) without epiphora (Control group) were injected 0.5 mL normal saline (NS).

A coin flip was utilized to randomly determine which eye was to be given the injection in the epibulbar area (Figure 3) and the contralateral was then included in the subpalpebral (Figure 4) group. Two calves with unilateral epiphora were excluded from the study and given treatment separately.

The detailed clinical examination and the vision tests were repeated on day 3 and day 7 in all the calves included in the study. Student's T test was applied to the Schirmer tear test and reduction percentage in the periocular matting values between the two routes of the drug administration. The significance was set at $P < 0.05$.

RESULTS

All the calves included in the study were free from systemic disease and were not on any medication.

The impaired vision was not noticed in any of the calves despite 41% (14/34) of them showing epiphora simultaneously. The superficial eye structures including eyelids, conjunctiva, cornea and sclera all appeared normal. Symptom's indicative of pain or irritation in and around the affected eye/s was also not observed in any of the calves throughout the study period.

The overflowing tears matting the periorbital hair were colorless (serous) in all the calves. In the calves of the treatment group, the overall average distance traversed by tears in the periorbital skin was 4.8 cm (range 1.0 cm to 13.0 cm) on day 0. In the eyes given subsequently injection in the palpebral subconjunctiva, the tear over-flow was 4.92 ± 0.59 cm (mean ± s.e) and in those injected by bulbar route 5.17 ± 0.90 cm.

STT values (27.67 ± 3.30 mm/min) in epiphoric were higher than non-epiphoric (21.00 ± 0.97 mm/min) eyes but the difference was not statistically significant ($P > 0.05$).

All the conjunctival swab samples showed similar pattern of growth with mixed type of colonies. However, the samples collected from calves with epiphora showed more number of colonies in comparison to those with no epiphora. Upon Gram's staining, both positive bacilli and cocci and negative bacilli (slender and stout) were observed. The bacterial growth seemed "commensals" only. *Morexella bovis* or *Morexella* like organisms were not noticed in any of the samples.

Direct bilateral ophthalmoscopy revealed no abnormality in any of the calves included in the study. The fundus reflection was clear with no shadow's indicative of the alterations in the superficial structures. The surface of optic disc was flat, and its edge visible. Vasculature was normal and abnormalities like edema, hemorrhage, exudation or irregularities in the retinal pigment were not noticed.

The subconjunctival injections were performed easily in the recumbent animals. The use of the insulin syringe was suitable for low volume withdrawal of the drugs from the vials. Its short, narrow gauge, permanently attached needle made possible safe delivery of the drugs in the desired location. The bleb that developed while depositing the medicine in palpebral or bulbar conjunctiva subsided automatically within a day in all the calves. In six calves, the epiphora subsided within three days. Their periorbital skin was dry. The average wet area had reduced to 2.3 cm on the right and 1.7 cm on the left side. The average matted area extended to 2.3 cm around eyes given palpebral subconjunctival injection and 1.4 cm in those injected via bulbar route. The reduction of facial matting values (mean ± s.e) was 65.5 ± 16.4% in eyes given epibulbar injection and 52.7 ± 22.4% in those administered the drugs via subpalpebral route. The P value was 0.648 and the values between the routes of drug administration showed no significant ($P > 0.05$) difference.

On day 7 post treatment, the epiphora had subsided completely in ten calves. In the remaining two animals, the matting was

noticed up to 2.0 cm and 1.0 cm (subpalpebral group) and 2.0 cm and 3.0 cm (epibulbar group) respectively. As compared to the day 0 values, the reduction in facial matting values (mean ± s.e) was 98.9 ± 7.1% and 86.1 ± 10.9% in the epibulbar and the subpalpebral groups respectively. The P value was 0.616. The values between the routes did not differ significantly ($P > 0.05$).

Calves in the control group neither showed epiphora nor any other abnormality subsequent to the subconjunctival injection. Those given treatment also showed no recurrence or deleterious effects.

DISCUSSION

Majority of the dairy cows maintained in this university farm belong to Jersey breed. Few Holstein Frisian cows are also reared. Consequently, Jersey calves were therefore disproportionally represented more in this study.

The epiphora developed in calves only. The heifers, adult dry and milking cows housed in separate pens, although grazing in different locations but using same highly dusty paths within the farm showed no epiphora or any ophthalmic condition during the study period.

The overflowing tears were colorless (serous) in all the affected animals. Unlike commonly encountered microbial infections, the tears were neither purulent nor accompanied by ocular pain, corneal opacity and ulceration[3, 8]. The reflex tearing might have protected the calves from the adverse ocular surface effects of the dust[5].

The severity of the epiphora assessed tentatively from the matted facial hair did not differ significantly between eyes and routes of subconjunctival injection; an important criterion for evaluation of the treatment protocol was thus fulfilled.

Schirmer tear test (STT) measures the quantity of tearing within a given time frame[9]. Recording for one minute gives satisfactory results in cattle[1, 10]. First described by Otto Schirmer in 1903, it is the fundamental diagnostic test used in veterinary ophthalmology[11, 1]. Tear production must be assessed before any agents have been instilled in the eye to prevent falsely elevated values[1]. The mean SST value obtained in our calves with no epiphora was within the normal range (> 20 mm/min) reported in this species[10, 13]. The values (27.67 ± 3.30 mm/min) obtained in calves with epiphora were non-significantly ($P < 0.05$) higher. Statistically significant difference is expected if larger number of calves can be included in such a study. The test was well tolerated by the calves and may be useful for evaluation of epiphora in calves.

The bacterial growth representing both gram positive and gram negative organisms with greater number of colonies developing from the swab samples obtained from our calves with epiphora seemed commensals only. It is difficult to ascertain the predominant organism with direct swab. Whether the composition or the immunological regulatory behavior of the microbiota residing on the ocular surface is affected by pollution is also not clear[14].

Direct ophthalmoscopy revealed no retinal abnormality in any of the eyes in our calves. In cattle the fundus examination plays an important role in diagnosis of several diseases like hypovitaminosis A[15]. and consumption of moldy corn[16].

Prompt treatment of the eye affections is essential. Drugs may be delivered to the eye in several ways: subconjunctival injection, topical application and systemic administration. Due to the constant turnover of the precorneal tear film, the drugs applied topically are soon washed out of the eye. To be effective, their frequent application may not only be stressful to the animal but also laborious and time consuming for the personnel[17].

The subconjunctival technique has the advantage of efficacy at a lower antibiotic dose[18]. It allows drugs to bypass the epithelium, one of the main barriers that limit drug entry[17]. Increase in drug absorption is also accompanied by prolonged contact time. Medications leak onto the cornea from the injection site and diffuse through the sclera into the globe[19]. Drugs with low solubility such as corticosteroids may provide a repository of drug lasting days to weeks[20].

In order to deposit the solution at the desired subconjunctival site without backflow or spillage and to prevent inadvertent needle punctures to the vital eye structures, the calves included in this study were restrained in lateral recumbency. The ophthalmic examination of an adult cattle restrained in standing is a challenge and the attempts commonly made to give subconjunctival injections may be dangerous particularly in the fractious ones[21].

In the calves of both the groups, similar volume (0.5 ml) and quantity of the drugs were administered subconjunctival. 0.5 mL per site is usually safe and effective in small animals[20]. Adult large animals are generally given up to 1.0 ml. Therapeutically effective tear concentrations are maintained for 24 hours or longer[18]. At all occasions, visible bleb developed at the site of injection indicating proper placement of the needle[19].

Gentamicin - dexamethasone combination was used to manage epiphora in all the calves included in this trial. The dose of gentamicin and dexamethasone used in our calves was less than that recommended for adult cattle[19]. The corticosteroids in the form of ophthalmic ointments or subconjunctival injections are commonly used and are effective in acute inflammatory conditions; whether the etiology is allergic, traumatic, or infectious[14, 22]. Antimicrobial agents like chloramphenicol, gentamicin, neomycin and sulphonamides are the drugs of choice in ocular therapy[14]. Although the eyes in the affected calves included in this study showed no pathognomonic symptom (except epiphora) of any microbial infection of clinical significance, the Gentamicin was given to prevent flareup of infection under corticosteroid administration. Steroids reduce resistance to many types of such infections except simultaneously with an effective antibiotic or other antibacterial medications[22].

The results of our study indicate that single dose subconjunctival injection of gentamicin - dexamethasone combination is effective in managing epiphora in calves. Subconjunctival injections allow drugs to bypass the epithelium; the barrier that limits their entry[23]. This route of administration is indicated for the treatment of lesions in the cornea, sclera, anterior uvea and vitreous. Animals given injection via subpalpebral or epibulbar conjunctiva showed no significant difference in their efficacy. In contrast to our findings, notable differences in efficacy of penicillin administered by these routes in calves with IBK was reported[18, 24]. However, in IBK appreciable involvement of several eye structures requires wide and deep spread of the drugs injected.

Calves in the control group did not develop any complications. Adverse reactions were not reported in calves with IBK, given clindamycin or isotonic saline solution subconjunctival[23].

No new cases or recurrence in calves included in the study may

be attributed to the moderate rainfall nine days later and settling of the excessive dust.

CONCLUSION

The results suggest that excessive dust in the environment may be associated with the development of serous epiphora in young calves. Subconjunctival administration of gentamicin - dexamethasone combination via the palpebral and bulbar routes are both effective for managing the ailment. However, more studies are needed to assess the deleterious ophthalmic effects of air pollution in the food animals so that various preventive or protective measures could be designed.

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Conflict of Interest
The authors declare no conflict of interest related to the study.

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