In the Eye of the Dawn

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Introduction

Squamous cell carcinoma is the second most common tumor in the equine, with sarcoid tumors being the most common[9]. However, squamous cell carcinoma is the most common tumor of the eye and associated adnexa[9]. There are several factors that have been associated with the development of squamous cell carcinomas, including breed and exposure to solar radiation. Draft breeds are more likely to develop ocular squamous cell carcinoma as well as light coated horses such as Appaloosa and Paints[9]. These tumors are most often diagnosed at an average age of greater than 10 years[9]. Diagnosis is made through excisional biopsy with histopathology. There are wide variety of treatment options including surgery, chemotherapy, and photodynamic laser therapy.

History and Presentation

Whiskey's Delta Dawn is a twenty-six-year-old Spotted Saddle Horse mare that presented to the Mississippi State University College of Veterinary Medicine Equine Medicine and Ophthalmology Services on February 11, 2020 for a growth on her right eye. Dawn's owners noticed a pigment change of the iris and increased cloudiness of the cornea approximately a few months prior to presentation. In the previous six weeks, Dawn had developed a thick, white ocular discharge and a growth was noted on the surface of the eye. The owners noticed that when approached from the right side Dawn would startle, but she had no difficulty navigating her pasture or other surroundings. No treatment for her ocular issues had been attempted by either the owners or their veterinarian. She was being treated with hydrochlorothiazide for a severe nosebleed after an attempted nasal endoscopy by the regular veterinarian. Dawn was last vaccinated in 2016 but was dewormed in early fall of 2019 with an unknown dewormer. She is housed on a property with six other horses and a donkey.

On initial physical exam, Dawn weighed 458 kilograms. She had a heart rate of 36 beats per minute, a respiratory rate of 24 breaths per minute, a rectal temperature of 99.7 degrees Fahrenheit, and a body condition score of 6/9 with 5 being ideal. She was bright and alert with normal mentation and hydration status. Mucous membranes were pink and moist with a capillary refill time of less than two seconds. On thoracic auscultation, no murmurs, arrythmias, crackles, or wheezes were noted. She had none to slight digital pulses in all four limbs. Normal borborygmi was noted in all four quadrants of the gastrointestinal tract. She was noted to have a large bony callous over the bridge of the nose from the level of the medial canthus of the eyes to the top of the muzzle, which was due to a traumatic injury two years prior per the owners. The left mandibular lymph node was enlarged when compared to the right. On ophthalmic exam, Dawn exhibited enophthalmos, blepharospasm of right eye, raised fleshy plaque-like mass on cornea with the largest part on ventromedial aspect and dorsolateral aspect near limbus. The mass did not appear to involve the conjunctiva. The left eye was noted to have an incipient cataract. At this time, a presumptive diagnosis of ocular squamous cell carcinoma was made based on appearance, but a superficial keratectomy was planned for excisional biopsy to confirm the diagnosis.

Diagnostics and Surgical Approach

On February 12, Dawn was sedated for a series of skull radiographs to investigate the cause of epistaxis from the previous endoscopy and fully examine the structure of the bony callous of her nose. The radiographs showed evidence of chronic periostitis, which was expected from the historic trauma, but emphysematous osteomyelitis could not be excluded. Two small ovoid structures were observed in the right rostral maxillary sinus which were suspected to be

abscesses or cysts, but neoplasia could not be ruled out. These structures were not investigated during Dawn's stay at MSU-CVM. Periodontal disease and malocclusion were also observed.

On February 13, Dawn was sedated for a standing superficial keratectomy of her right eye. The right side of the face was clipped and prepared with dilute betadine and saline. Regional anesthesia was applied to block the innervation to the right eye. The cornea was incised using a beaver blade and a Martinez corneal dissector was used to complete the keratectomy. A lateral canthotomy was performed to allow further dissection of the corneal mass. Westcott tenotomy scissors were used to excise a conjunctival mass at the lateral limbus. A small area at the ventral limbus was also removed and submitted for histopathology. Once the keratectomy was complete, the exposed area was painted with 0.5mL of Emundo® indocyanine green dye. A diode laser was used to emit five cycles of 15 Joules were used per quadrant of the eye for a total of 289 Joules on the cornea. The lateral canthotomy was closed in a two-layer closure. A subpalpebral lavage system was placed in the dorsal eyelid in order to treat with topical atropine and triple antibiotic solution. The resected cornea and conjunctival specimens were submitted for histopathology. Histopathology confirmed the diagnosis of ocular squamous cell carcinoma.

Pathophysiology

Squamous cell carcinoma is a malignancy of superficial epithelial structures, meaning that this type of tumor can arise from any squamous epithelium of the body. Squamous cell carcinomas have been diagnosed in numerous species. In horses, squamous cell carcinoma has been diagnosed in the eye and adnexa, genitalia, face, pinna, perianal region, extremities, and stomach[14]. Squamous cell carcinoma is the most common neoplasia of the equine eye and associated structures[9]. Squamous cell carcinoma may affect any and all parts of the eye, including the cornea, conjunctiva, nictitating membrane, and eyelids[9,14]. This tumor type is

reportedly slow to metastasize, but can be locally invasive, often causing blindness[7,10]. Metastatic rates are between ten to fifteen percent and lesions often appear in regional lymph nodes but can also involve the salivary glands or thorax[7,9,10]. Historically, the cause of squamous cell carcinoma was thought to be influenced by skin pigmentation and chronic exposure to ultraviolet radiation. Although these factors play a role, more recently it has been evidenced that an equine papillomavirus, equine caballus papillomavirus-2 (EcPV-2), is a more causative agent of squamous cell carcinoma formation[14]. EcPV-2 DNA has been identified in 100% of ocular and genital squamous cell carcinomas[14]. For years, there has been a known link between bovine papillomavirus and equine sarcoid formation. Like with sarcoid formation and bovine papillomavirus, horses exposed to EcPV-2 will not necessarily develop squamous cell carcinoma, but the infection is required for the horse to be able to undergo squamous cell carcinoma formation[14]. The recent link between EcPV-2 infection and squamous cell carcinoma diagnosis allows for a greater understanding of the tumor occurrence and pathogenesis.

Draft breeds as well as light colored breeds (Appaloosa, Paint, Pinto, etc.) are predisposed to squamous cell carcinoma formation[5,13]. Genetic links within some affected breeds have been discovered. A missense variant of the damage-specific DNA binding protein 2 (DDB2) has been associated with development of ocular squamous cell carcinomas in several breeds, including Belgians, Haflingers, Rocky Mountain Horses, and Connemara Ponies[2,5,13]. DDB2 works to repair DNA that has been damaged by ultraviolet radiation[5,13]. When the variant form of this gene is present in either heterozygous or homozygous individuals, ocular and periorbital structures damaged by solar radiation are more likely to form squamous cell carcinomas, as they cannot be repaired appropriately[2,5,13]. The expression of this genetic defect is autosomal recessive, but the allele frequency is high within these breeds, therefore allowing for

many homozygous recessive (affected) animals[2,5,13]. The allele frequency is extremely low in other highly affected breeds, mainly Appaloosas and Paints, therefore another genetic cause or a combination of other causes should be investigated to determine the etiology of squamous cell carcinoma development in these breeds[2,5,13]. Due to the association of this genetic defect and solar radiation, UV protective fly masks and stalling during peak hours of sunlight are suggested to reduce incidence of tumor formation in all breeds[13].

Treatment Options

Surgery has always been the mainstay of treatment for equine ocular squamous cell carcinoma. The degree of involvement of the ocular or periorbital lesion determines the type of surgery required[9,14]. In Dawn's case, a superficial keratectomy was performed to debulk the mass and remove as much as visibly possible. Greater margins were taken around the limbus to ensure the entire tumor had been removed. However, entire tumor resection of many ocular squamous cell carcinomas is not possible due to the local invasiveness[9,14]. The anatomy of the region often prevents full surgical removal of the tumor[9,14]. These physical barriers have brought about the necessity of adjunctive therapies to pair with surgical intervention. In a retrospective study over 17 years, recurrence rates of ocular and periorbital squamous cell carcinomas were recorded[12]. Patients were treated with surgery or with surgery and radiation therapy[12]. Those patients that underwent surgery alone were significantly more likely to have tumor recurrence, regardless of lesion location[12].

Chemotherapy is the most common adjunctive therapy used in combination with surgery. Several chemotherapeutic agents have been tested for effectiveness against squamous cell carcinomas. The most common agents used are cisplatin or 5-Fluorouracil, but mitomycin C and bleomycin have also been used. Cisplatin, or cis-diamminedichloroplatinum, is a chemotherapy

agent that uses its platinum molecule to crosslink purine bases within DNA and disrupt the cell cycle resulting in cancer cell apoptosis[6]. Cisplatin is used in periorbital squamous cell carcinomas as an intralesional injection or with impregnated beads placed under the skin[9]. 5-Fluorouracil, or 5-FU, is a pyrimidine analog that interferes with nucleoside metabolism and results in cytotoxicity and cell death[15]. 5-FU is used as a topical cream or solution. Mitomycin is an antimicrobial drug that has anti-tumor properties[11,14]. It is less commonly used, but has been used as a sole agent when surgery is not an option[11]. Bleomycin is another antimicrobial with anti-tumor properties, but in a study comparing it to cisplatin, bleomycin was not as effective and it is more expensive[14]. Therefore, bleomycin is not commonly used in the treatment of squamous cell carcinomas. There has been a wide variation in the success rates of chemotherapeutics. Much of the success is dependent on tumor location and size. In smaller and more localized tumors, there is higher success of remission, but in larger more involved tumors, recurrence rates are higher[14]. This was independent of the chemotherapeutic agent used.

Cryotherapy is another commonly used technique that is much more available to the general practitioner. Cryotherapy involves the application of liquid nitrogen (or less commonly nitrous oxide) to the affected area after surgical debulking[3]. Depending on the location of the tumor, a closed probe or spray application can be used. A double cycle of freezing and thawing should be used to ensure maximum results[3]. Cryotherapy works under the principle of cryonecrosis, which is the formation of intracellular ice formation which leads to apoptosis of cells[3]. Unlike chemotherapy, cryotherapy does not discriminate between rapidly dividing cancerous cells or healthy surrounding cells. Thus, application of liquid nitrogen should be precise over the tumor area and margins, so as to not damage excessive amounts of non-diseased tissue[9].

Radiation therapy has historically been available at some veterinary facilities. Radiation therapy with beta radiation involves the application of strontium-90 using a probe directly to the lesion[9]. Strontium-90 has limited penetration into tissues therefore surgical intervention is necessary prior to application[9]. Radiation with strontium-90 has a high likelihood of causing corneal ulcers that have a significant delay in healing and can also result in bacterial keratitis[9]. Another form of radiation therapy is referred to as brachytherapy. Brachytherapy involves continuous exposure to radioactive material over a period of time[9]. This is done using a variety of radioactive molecules, such as cesium-137, radon-222, gold-198, cobalt-60, and iridium-192[9,14]. Beads of these radioactive materials are placed within a tube and the tube is inserted within the lesion of an adnexal tumor, but cannot be used to treat corneal lesions[9,14]. The disadvantages of this therapy include cost, access to required techniques and skill, radiation exposure of personnel, necessary isolation of the horse, and local tissue necrosis and infection[9]. Radiation therapy has been historically very successful with and without surgery, however access to institutions willing to handle hazardous materials is becoming more difficult[14].

Photodynamic therapy is the most recent addition to the arsenal to fight ocular squamous cell carcinomas. It has been used previously for a wide variety of diseases in human medicine including cancer and resistant bacterial infections[4]. Photodynamic therapy uses light and a light-sensitive compound to cause cell necrosis while in an oxygen-rich environment[8]. In small animals, the light-sensitive compound is injected intravenously, and the diode laser is applied to the area of interest[4]. In horses, the volume of compound that would be required for intravenous therapy is too great, therefore methods of either intralesional or topical application have been developed[4]. There are a few light sensitive compounds that are available, but all work in similar mechanisms. These light sensitive compounds work by diffusing into the cancerous cells and

when the diode laser is applied, the compound reacts with oxygen to create reactive oxygen species and free radicals that cause cellular damage and cause apoptosis[1]. Photodynamic therapy may also alter the vascular structure with tumors, may cause vascular leakage or damage, and may cause a release of cytokines producing a local inflammatory response[1]. The success rates of the initial literature reports of using photodynamic therapy as an adjunctive therapy for ocular and periorbital squamous cell carcinomas have been very successful with a 100% remission rate at a minimum of two years in a pilot study[8]. When compared to other adjunctive therapies, photodynamic therapy is highly advantageous as it requires less hospital visits when compared to cisplatin therapy, access is more available as the diode lasers are more cost effective for general practitioners or tertiary hospitals than radiation materials, hospital stays are more limited, and general side effects of therapy are more limited[8]. The main disadvantage of photodynamic therapy currently is that many general practitioners are not aware of its availability and wide variety of uses.

Case Outcome

After surgery, Dawn was hospitalized until February 19th. She was maintained on NeoPolyGram eye solution every 8 hours and atropine solution every 24 hours though the subpalpebral lavage, flunixin meglumine every 12 hours intravenously, and trimethoprim sulfadiazine every 12 hours orally. She was discharged with the NeoPolyGram, atropine, and flunixin meglumine and scheduled for a recheck examination in two weeks.

Dawn presented to MSU-CVM again on March 3, 2020 for her scheduled recheck exam. Two days prior to her appointment her right eyelids became swollen and copious amounts of white purulent discharge were noted. Her owners had kept her stalled for the two weeks between discharge and representation but were not protecting the eye with a fly mask. The owners ran out

of flunixin meglumine two days prior and switched to giving oral doses of phenylbutazone to help with inflammation and pain. Dawn had been eating and drinking well at home. Dawn's physical exam during her recheck appointment was similar to her exam on presentation. She still had the enlarged left mandibular lymph node but was otherwise normal. On ophthalmic examination, mucopurulent discharge, excessive lacrimation, along with marked chemosis of the inferior eyelid were noted in the right eye. The subpalpebral lavage was still intact. The left eye was unchanged from previous exam. Cellular corneal infiltrate was seen in the right cornea. This area was swabbed for cytology, which revealed a bacterial infection. The area was then swabbed further for bacterial culture and sensitivity and fungal culture to determine the etiology of the keratitis. While results were pending, Dawn was started on Cefazolin 3.3% solution every 2 hours, Ofloxacin 0.3% solution every 2 hours, Voriconazole 1% solution every 4 hours, and atropine 1% solution every 24 hours, through the subpalpebral lavage. Dawn was restarted on flunixin meglumine intravenously as well. Dawn was fitted for a mask to protect her eye from any trauma and excessive sunlight. Results of the cultures revealed infection with Streptococcus spp. and Staphylococcus spp. but no growth of fungal organisms. The bacteria were susceptible to the antibiotics already prescribed therefore they were continued. Dawn remained in hospital for ten days as medications were given every 2-4 hours. Her ulcer continued to heal and on day 10 of medical therapy, Dawn was discharged with instructions to keep her in the mask with her eye covered until her keratectomy site had fully healed. Her corneal ulcer was much smaller at the time of discharge. She was discharged with topical cefazolin, voriconazole, ofloxacin, and atropine, as well as flunixin meglumine orally and planned to recheck in two weeks to assess healing. Unfortunately, due to COVID-19 restrictions, Dawn's appointment was cancelled, and her owners were instructed to follow up with their regular veterinarian.

Conclusion

Squamous cell carcinomas are common skin tumors of horses, and the most commonly reported tumor of the cornea and periorbital tissues. Breed predispositions are known, but further investigation remains to fully divulge the etiology of this tumor in certain breeds. Diagnosis is straightforward using excisional or incisional biopsies and histopathology. Treatment options vary depending on location of the tumor and capabilities of the treating veterinarian. Surgical intervention remains a mainstay of curative therapy, but adjunctive therapies are paramount in decreasing the incidence of recurrence. Newer adjunctive therapies offer hope of better and longer cure rates, without increasing the burden of cost or side effects.

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