Conjunctival Biopsy of Non-Infectious Conjunctivitis in Three Psittacine Birds

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Three cases are presented to show the usefulness of conjunctival biopsy as a method of diagnosing non-infectious conjunctivitis. The lorikeet and *Agapornis* sp biopsy samples were taken under isoflourane anesthetic with a simple wedge incision on the conjunctiva. In both cases there was a moderate amount of bleeding after biopsy which continuing post-anaesthesia. In Case 3 (budgerigar) an endoscopic 5 fr. grasping biopsy forceps were utilised under isoflourane anesthetic and no bleeding was observed. All three cases showed that although antibiotics may be beneficial for secondary problems, each of the cases would have benefited from earlier diagnosis utilising conjunctiva biopsy.

CASE 1

Conjunctival squamous cell carcinoma. Five year old female *Agapornis sp.* peachface lovebird

Presented for: Sudden onset of conjunctivitis
History:
Continual megabacteriosis (*Macrorhabdus ornithogaster*) for two years despite oral amphotericin. Annual health check for 3 years, negative for *Chlamydophila* antibodies.
Treatment:
Initially doxycycline injection and anaesthetise to investigate and remove, clean and debride conjunctiva. Foreign body-negative. Tetracycline eye cream, amoxycillin clavulanic acid drops 125mg/kg PO q12h for 10 days. Unable to get worthwhile sample for culture and sensitivity.

Day Five
Pus decreased, sinus swollen – flushed out sinus with saline and performed culture and sensitivity. Doxycycline injection 50mg/kg IM, amoxycillen clavulanic acid drops 125mg/kg PO q12h for 10 days and gentamycin 50mg/kg drops in nostril q12h.

Day 15
Eye swollen, examined under anaesthetic, possible nodular mass on the conjunctival membrane on medial side of upper eyelid. Tried to resect but incomplete removal. On culture results, place on oral trimethoprim/sulphonamide 96mg/kg PO q12h.

Tests performed:
Culture and sensitivity
Specimen:
Eye swab
Test results:
Gram stain:2+ gram positive cocci1+ gram negative bacilli

Culture:Heavy growth of *Enterobacter sp.* [1]Heavy growth of *Enterococcus sp.* [2]
Isolate 1 sensitive to cotrimoxazole, neomycin, tetracycline, enrofloxacin, lincospectin, framycetin and doxycycline. Resistant to cephalexin and clavulox.
Isolate 2 sensitive to cotrimoxazole and lincospectin. Resistant to clavulox, neomycin, tetracycline, enrofloxacin, framycetin and doxycycline.

21 days later
Biopsy using wedge resection under isoflourane anaesthetic:
Gross pathology: Two irregular fragments of pale tan resilient tissue, each measuring approximately 2 x 1 mm. Also included is an irregular piece of tan resilient tissue measuring 1 x 1 mm.
Histopathology: There is a conjunctival tumor arising from infiltration of the lamina propria by broad anastomatic trabecular formations of neoplastic, mildly plemorphic, occasionally mitotically active squamous cell contiguous with overlying neoplastically transformed epithelium and featuring multifocal dysplastic keratinisation. Neoplastic tissue extends to the margins of the fragmented biopsy material.

Diagnosis:
Conjunctival squamous carcinoma. Complete excision, if this can be achieved, would be expected to give a favourable prognosis.

Result:
Died two months later.

Usefulness of biopsy:
Multiple medications used that may have been avoided.

Culture and sensitivity performed that delayed diagnosis, as suggested conjunctivitis was infectious. Earlier biopsy would have solved problem and decreased bird's pain and suffering.

CASE 2

Possible allergic or irritation of conjunctiva post infection or inflammation in a six-month old rainbow lorikeet

Presented for:
Watery irritated painful conjunctiva and eye but not inflamed conjunctiva, with feather colour changes around eye.

History:
Bought from bird shop two months previously. Outside aviary in contact with circovirus positive rainbow lorikeet in aviary.
Owner renovating and removing asbestos and lead from house. Very dusty during this period.

Clinical examination:
Right eye painful to touch and refused to keep open but not red or inflamed. Feathers wet around eye and have turned purple.

Treatment:
Doxycycline injection 50mg/kg IM and enrofloxacin 25 mg/kg PO q12h for 10 days.

Tests performed and results:
Crop: gram 90% large gram-negative rods 10% gram-positive fat rods.
Faecal fresh, negative gram stain, occasional gram-positive rods and gram-positive cocci.
Chlamydophila antibody test negative 0
Circovirus PCR feathers negative

One week later eye still closing and watery, bird very quiet.

Further tests:
1) *Culture and sensitivity*
   Specimen: Choana and conjunctiva
   Test results:
   Gram stain: occasional gram positive cocci
   Culture: bacteria not isolated after 24 or 48 hrs
2) *Biochemistry and haematology*:
   serum gluc 9.4, urea 0.6, ca 2.3, prot, total 26, alb 12, glob 14, a:g ratio 0.9, ast 240, ck 2185, chol 4.5, amyl 631, gldh 1, bile acids postprandial 8, uric acid 0.1, pcv 42%.
   Total white cell count 45000
   Heterophils 87%, lymphocytes small 7%, monocytes 6%
   Heterophils all toxic or immature. Lymphocytes all small and normal but low numbers.

3) R*adiographs* no significant findings.

Treatment:
Doxycycline injection 50mg/kg IM, oral itraconazole10mg/kg oral q24h, enrofloxacin 25 mg/kg PO q12h for 10 days and Cloxacillen eye ointment topically on the eye.

Owner now mentioned history of access to lead due to ongoing house renovation so calcium edta 40mg/kg IM twice weekly for 2 weeks.

Two weeks later:
No change, eye still closing, watery discharge.
Tests:
Haematology PCV= 45%
Total white cell count 42000, heterophils 87%, lymphocytes, 13% platelets ok, no monocytes seen.
Biopsy using wedge resection under isoflourane anaesthetic:
Gross pathology: Three fragments of pale tan tissue measuring less than 5 mm across.
Histopathology: Both conjunctival biopsies are covered by pseudostratified cuboidal conjunctival epithelium. The submucosa is well vascularised and mildly oedematous. There is a mild to moderate infiltrate of the epithelium and superficial submucosa with heterophils accompanied by low numbers of lymphocytes and plasma cells.
Small biopsy of eyelids is covered by stratified squamous epithelium. There is a mild to moderate infiltrate of the submucosa with heterophils and lower numbers of lymphocytes and plasma cells.
Diagnosis:
Suppurative conjunctivitis, eyelid suppurative dermatitis.
Pathologists Comments:

There is a mild to moderate suppurative conjunctivitis and dermatitis. The aetiology of this lesion is not apparent however, it may be associated with a bacterial infection or sterile inflammation secondary to chronic irritation (e.g. dust)

Treatment:
Maxidex (dexamethasone 1mg/ml eye drops) applied to the eye four times in 24 hours. Improved in twelve hours and still improved after 48 more hours. Bird's demeanor improved.

Result:
Improved with maxidex (dexamethasone 1mg/ml eye drops) eye drops q3h over 24 hours after six weeks of no response to antibiotics. At yearly health check one year later mild watery eye, suspect blocked tear duct post-inflammation, eye open, bird's demeanor greatly improved.

Usefulness of biopsy:
Corticosteroids drops are rarely utilised for conjunctivitis in avian species and are often contraindicated.
Multiple medication and diagnostic tests performed.
Biopsy allowed diagnosis of post-infection/irritation inflammation.

CASE 3

Conjunctival polyp in Adult Budgerigar

Presented for: Unilateral right eye conjunctivitis, all other signs normal.

History: Bought from auction by show budgie breeder.

Treatment: Doxycycline injection 50mg/kg IM
Doxycycline HCL powder 40mg/g in water 45 days.
Enrofloxacin25 mg/kg PO q12h for 7 days.
Owner declined all other tests and treatment. Advised to treat other three birds bought and in contact with doxycycline in water.

Week 4 No change. Enrofloxacin25 mg/kg PO q12h for 10 days, meloxicam oral 1.5mg/ml q24h for 7 days and Elizabethan collar.

Week 5
No change in eye but has megabacteria on faecal.
Biopsy of conjunctiva performed under isoflourane anaesthetic using endoscopic 5 fr. grasping biopsy forceps. No bleeding observed during or post biopsy.

Biopsy:
Two 1 x 1 mm samples from conjunctival mass using endoscopic 5 fr. grasping biopsy forceps.
Histopathology: Congested. Prominent vessels. Surface folded in frond-like manner. Epithelium cuboidal. Resembles a papillomatoid-like projection. Not an active conjunctivitis.

Conclusion:
Conjunctivitis , Papilloma.
Pathologist notes:

This small mass is proliferative with a dense core of small vessels. The overlying mucosal epithelium is hyperplastic with occasional mitotic figures and the cells are more cuboidal than expected. Although described as papillomatoid, that is a description of the formation, not necessarily the aetiology.
Result**:**
Removal and resolution of conjunctivitis.

Usefulness of biopsy:
Able to place birds with other birds without concern of infection.
Diagnosis prevented major follow up diagnostic tests and clinical exams on flock for possible Chlamydophilosis or viral concerns.
No further medications necessary.
Utilisation of flexible endoscopic 5 fr. grasping biopsy forceps showed samples can easily be taken without haemorrhage.

Conclusions

- Conjunctivitis does not always have an infectious cause.
- Infection present, and culture and sensitivity did not rule out infection may be secondary or primary with secondary problem arising.
- Biopsy is useful tool for determining cause of non-antibiotic responsive conjunctivitis.
- Biopsy performed earlier in these cases would have reduced pain to the bird and saved money for the owner.
- Biopsy using the small endoscope 5 fr. grasping biopsy forceps was easily performed with no bleeding.
- Possible in future to do biopsy using 5 fr. grasping biopsy forceps under local anaesthetic with or without gaseous sedation and without hospital stay.
- Possible that many cases of both of infectious and non-infectious conjunctivitis could be cost effectively diagnosed and therefore resolved earlier if conjunctival biopsy had been utilised.

Discussion

Conjunctival biopsy is widely utilised and reported in the mammalian species. Reports of diagnosis of conjunctivitis by biopsy in avian species are not uncommon. However they generally are of infectious conjunctivitis.

Most textbooks suggest various tests and treatment trials, and usually recommend biopsy as almost a diagnosis of last resort. This is despite testing (including Chlamydophila, haematology, impression smears of the eye, culture and sensitivity) and treatment trials may be less cost-effective than performing a biopsy.
In contrast, veterinary ophthalmologists are more likely to perform a biopsy in the first consultation for a severe conjunctivitis of infectious or non-infectious nature.

Non-infectious conjunctivitis is not necessarily uncommon but is poorly reported in the literature. Neoplasia has been reported in a few psittacines including a xanthoma in a budgie and a basal cell carcinoma in a conure. Allergies, irritants and vitamin A deficiency lesions have also been sporadically and tenuously reported.

Many conjunctival biopsies are performed using a wedge technique. Although bleeding would be expected from this, bleeding was small to moderate. The use of the 5 fr. grasping biopsy forceps was successful in taking samples without bleeding. These 5 fr. grasping biopsy forceps are now utilised at the Bird & Exotics Veterinary Clinic for Biopsy sampling of the conjunctiva, oral cavity and skin of all exotic mammals, reptiles and birds.

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