Feline ear polyps: Two case reports and a model for pathogenesis – chronic otitis media with effusion

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SUMMARY

Inflammatory ear polyps are the most common non-neoplastic masses in the middle ear of the cat. These polyps tend to occur in younger cats. The two cases described in this paper show that ventral bulla osteotomy is a promising surgical option to successfully manage the condition and prevent recurrence. Cytologically, these polyps show all the characteristics of a chronically inflamed middle ear mucosa, i.e. massive lymphocytic and purulent inflammation as well as metaplasia of the mucoperiosteum displaying ciliated columnar epithelium and goblet cells. Impression smears of the polyps also show lymphocytes in follicular arrangement. Cytology is a suitable method to quickly obtain a preliminary intraoperative diagnosis. However, this initial diagnosis needs to be confirmed by histological examination of biopsy samples. Chronic otitis media with effusion (COME) is a possible cause of the disease. It can also serve as a model to explain the pathogenesis of inflammatory auro-nasopharyngeal polyps in the cat.

Abbreviations: COME = chronic otitis media with effusion; MALT = mucosal-associated lymphoid tissue; VBO = ventral bulla osteotomy

Keywords: Otitis media, ear polyp, cytology, metaplasia, cat


Introduction

Ear polyps are nodular and often pedunculated non-neoplastic masses, which originate from the mucosal lining of the middle ear and/or from the transition zone to the Eustachian tube [Pope and Constantinescu, 2000]. They tend to occur in younger cats [Donnelly and Tillson, 2004], but have also been reported in older cats aged up to 15 years [Faulkner and Budsberg, 1990; Veir et al., 2002]. Cats often suffer from otitis media due to viral infections of the upper respiratory tract [Venker-van Haagen, 2005]. Polyps are considered a late sequela of the chronicification of an inflammation of the middle ear [Salvinelli et al., 1999; Fan and De Lormier, 2004; Zender et al., 2007]. The middle ear is formed by the tympanic cavity and the embedded auditory ossicles. The major portion, the tympanic cavity, is a hemispherical bulla with a smooth surface, projecting above the base of the feline cranium. A medially protruding septum divides the cavity incompletely into a ventral and a dorsal compartment. The auditory tube, a narrow bony and cartilaginous channel, connects the middle ear to the nasopharynx [Seiferle, 1984]. The lining of the bony middle ear wall consists of modified respiratory squamous epithelium. The thin and poorly vascularised mucosa closely overlies the periosteum and has therefore been named mucoperiosteum [Paul, 2008]. The mucoperiosteum contains some ciliary and secretory cells as well as goblet cells [Seiferle, 1984; Jung HWA Pieper, 2003]. In the region of the promontory, on the medial wall of the middle ear...
ear, and particularly in the transition zone to the Eustachian tube, cuboidal to columnar stratified epithelium, with or without kinocilia, and goblet cells can be found. The transition from modified squamous epithelium to respiratory epithelium with kinocilia is continuous [Arnold, 1977; Harvey et al., 2003; Liebich, 2003]. The Eustachian tube is completely lined with stratified ciliated epithelium containing goblet cells [Liebich, 2003]. Inflamed mucoperiostium contains highly organized lymphatic tissue called MALT (mucosal-associated lymphoid tissue). In humans and in rats, it has been demonstrated that no lymphatic follicles develop in the normal, non-inflamed mucosal membrane of the middle ear. Lymphoid follicles require an external stimulus to develop, e.g. a viral or bacterial pathogen. Paul (2008) called this the “triggered dynamics” of the development of MALT.

Depending on the cause, and under the influence of pathophysiological conditions in the middle ear (partial pressure of carbon dioxide and oxygen), inflamed mucoperiostium undergoes muciparous or keratinizing metaplasia [Parker and Binnington, 1985; Kuipers et al., 1996; Esterline et al., 2005; De Lorenzi, 2005; Ressl and Poli, 2007; Paul, 2008; Rossi, 2009]. In addition, lymphatic follicles can always be found in the submucosa. Aural discharge, pruritus and pain are the most common clinical signs of otitis media. Some animals also show neurological symptoms such as head tilt, nystagmus, Horner’s syndrome or facial nerve paralysis. Up to one third of the patients develop deafness in the affected ear [Anders et al., 2008; Diel, 2008]. If inflammatory polyps reach as far as into the nasopharynx, animals display respiratory symptoms [Macphail, 2008; Wurtz-Tutschku, 2008]. In the literature, two methods have been discussed for removing ear polyps.

1) One option is the manual removal by traction-avulsion via the ear canal, a method, which is related to high recurrence rates [Anderson et al., 2000]. Recently, this technique has been further developed performing “perendoscopic transtympanic excision” [Mortellaro et al., 2001; Diel, 2008].

2) Ventral bulla osteotomy (VBO) is a surgical procedure to remove ear polyps and may produce complications e.g. Horner’s syndrome, vestibular disturbances, otitis media, haemorrhage, hypoglossal nerve damage, facial nerve paralysis and damage to the structures of the auditory apparatus. However, recurrence rates are lower after ventral bulla osteotomy [Donnelly and Tillson, 2004].

In humans, COME often occurs without perforation of the tympanic membrane or without otorrhoea and may therefore take an asymptomatic course. In cases with late clinical manifestation, sensorineurally induced reduction of the patient’s ability to hear, acute exacerbation of a chronic otitis media and complications regarding the bony structures may occur. Starting as a simple purulent, and at a later stage mucopurulent, inflammation, the condition may worsen producing granulation tissue (polyps) and cholesteatomas.

In most cases, the disease is caused by a viral infection of the upper respiratory tract and the resulting acute middle ear infection. Babies and infants are predominantly affected, particularly those who are cared for in nurseries.

The larger the number of children living together, the higher the incidence of upper respiratory tract diseases. Bacterial contamination as a sequela of an impaired auditory tube function and massive development of subepithelial follicles, serving as a histomorphological substrate, are perpetuating factors of chronic otitis media with effusion (COME) [Sautter and Hirose, 2007; Paul, 2008].

Inflammatory mediators also play an important role in the development of COME. Bacterial endotoxins perpetuate the condition by migrating into the inner ear where they cause a loss of auditory capacity [Juhn et al., 2008].

Material and methods

Cytology

A sample of the aural discharge is retrieved from the ear canal using a cotton swab. The collected sample is then rolled onto a glass microscope slide and stained using Haema Schnellfärbung® (quick stain) (Labor+Technik, Berlin, Germany) and evaluated under the light microscope. Intraoperatively, cells are obtained by direct impression smear and then stained using Haema Schnellfärbung® as well as modified May-Grünwald Eosin Methyline Blue® (Merck, Darmstadt, Germany) and Giemsa® (Merck, Darmstadt, Germany) before evaluating them under the microscope.

Imaging techniques

Lateral oblique and rostrocaudal radiographs are taken of the sedated patient, as described by Harvey et al. (2003).

Surgery


Anaesthesia and analgesia

To sedate patients for radiographic examination and to anaesthetize them prior to surgery, medetomidine at a dose of 0.05 mg/kg (Domitor®, Pfizer, Vienna) und ketamine (8 mg/kg (Ketamidor®, Richter Pharma, Wels) were given IM. Anaesthesia was maintained with isoflurane (Isoflo®, Abbott, Richter Pharma, Wels). Whenever necessary, a bolus of fentanyl (0.5 mg Fentanyl-Janssen®, Janssen-Cilag, Vienna) diluted in 100 ml 0.9 % saline solution (Natrium chloratum physiologicum®, Fresenius Kabi, Graz) was administered via the infusion line, up to a maximum of 0.25 ml per kg body mass per 5 minutes. Before and after surgery, patients were given carprofen (2 mg/ kg) (Rimadyl®, Pfizer, Vienna) as an analgesic.

Case details

Case 1

A 15-month-old castrated, regularly vaccinated, male Maine Coon cat with a body weight of 9.5 kg was presented with pruritus and pain in the right ear. The cat lived in a household together with one of his male litter mates. The second cat was asymptomatic. Clinical examination revealed unilateral purulent otitis. Differentials to consider include bacterial inflammation, Malassezia otitis, ear mange caused by Notoedres, allergic otitis and/or neoplasias in the external ear canal or the middle
ear. First samples were taken for smear preparations and the external ear canal was examined using an otoscope. Cytological findings revealed the presence of neutrophil granulocytes with large quantities of phagocytised cocci, consistent with bacterial otitis. Due to the copious purulent otic discharge, the tympanic membrane could not be evaluated. The cat received marbofloxacin 2 mg/kg s.i.d. PO (Marbocyl®, Vetoquinol, Vienna) for one week together with a topic treatment b.i.d. (0.05 % chlorhexidine otic solution; Clorexyderm®, ICF, Cremona, Italy). At follow-up examination, the volume of the otic discharge was significantly reduced. Haematological and blood chemistry findings were normal. The patient was sedated according to the previously described protocol and the ear canal was cleaned using diethylhexyl sodium sulfo succinate and urea hydrogen peroxide (Otoprof®, ICF, Cremona, Italy). The following otoscopic examination revealed a pink bulging mass in the region of the tympanic membrane; remnants of the ruptured eardrum could be discerned at the wall of the external ear canal. The patient was diagnosed with otitis media with eardrum perforation caused by an ear polyp.

Fig. 1: Oblique skull radiograph of the Maine Coon cat (Case 1). The healthy bulla (a) appears as a thin-walled dense bony structure at the base of the skull, while the affected right side (b) shows a soft tissue opacity in the tympanic cavity.

The soft palate was carefully elevated using a feline spay hook to visualize the pharynx, but no nasopharyngeal polyps were detected. Radiographic examination in oblique lateral projection showed a soft tissue opacity in the right tympanic cavity. The contralateral bulla appeared as a thin-walled dense bony structure (Fig. 1). The owner agreed to have the polyp removed using the traction-avulsion technique (Fig. 2). Samples for bacteriological culture and histopathological examination were obtained during surgery. Postoperatively, antibiotic therapy was started with amoxicillin-clavulanic acid (Clavaseptin®, Vetoquinol, Vienna) at a dose of 20 mg/kg b.i.d. PO. Bacterial culture proved the presence of enterococcus sp., and the antibiogram showed that the responsible organism was sensitive to the initial antibiotic treatment, so the therapy was extended to a total of one month. Histopathology revealed that only some regions on the external surface of the polyp were covered by one or two layers of cuboidal epithelium, while the majority of the surface was eroded (Fig. 3a). In addition, some adenoid indentations with columnar ciliated epithelium were seen (Fig. 4a). In the loose connective and granulation tissue of the polyp stroma, follicle-like accumulations of small lymphocytes can be seen. b) Lymphocyte follicular aggregates are also observed in the impression smear preparation (Haemacolor).

Fig. 3: Longitudinal histological section of the polyp of Case 1: a) In the loose connective and granulation tissue of the polyp stroma, follicle-like accumulations of small lymphocytes can be seen. b) Lymphocyte follicular aggregates are also observed in the impression smear preparation (Haemacolor).
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Follicle-like accumulations of small lymphocytes were found (Fig. 3b). The purulent inflammatory component was only mildly developed.

After only three weeks, the tympanic membrane was fully restored and closed, and the cat was discharged as healthy.

One year later, the cat was again presented, this time with bilateral nasal discharge and a unilateral purulent discharge from the right ear. After one week of pretreatment with amoxicillin-clavulanic acid and acetylcysteine 50 mg (ACC® Hexal, Hexal Pharma, Vienna) per meal twice daily, skull radiographs were taken revealing a soft tissue opacity. The opacity was interpreted as a regrowth of the ear polyp. The pharyngeal cavity was clinically normal.

This time, a ventral bulla osteotomy was the treatment of choice because this technique is associated with low recurrence rates. Due to the bone density of the ventral wall of the tympanic cavity in this Maine Coon cat, it was quite difficult to perform the osteotomy and achieve perforation of the bulla.

Again, samples for bacteriological and cytological examination were collected intraoperatively, and the removed polyp was submitted for histopathological examination. Bacteriological findings did not differ from those of the first examination.

At a magnification of x4, cytology was characterized by dark-blue cell accumulations consisting of numerous lymphocytes, plasma cells, many neutrophils, macrophages and phagocytised cocci (Fig. 3b). Outside these areas, columnar epithelium with (Fig. 4d) and without kinocilia (Fig. 4b) as well as some goblet cells were observed (Fig. 4c). Fig. 5 shows the stratified composition of the epithelia. Type and structure were compatible with metaplastic inflammatory ear polyps.

After one month of antibiotic therapy with amoxicillin-clavulanic acid (Clavaseptin®, Vetoquinol, Vienna) and once the eardrum had closed again, the cat was discharged as healthy.

Two years later, no regrowth of the polyp had occurred.

Case 2

A 10-month-old castrated European Shorthair cat with a body weight of 3 kg was presented with pruritus, pain and a yellow discharge in the right ear. The cat came from an animal shelter where she had contracted infectious rhinitis at the age of 8 weeks. She suffered from a severe nasal discharge and respiratory symptoms. At the age of four months, the cat was diagnosed with generalized dermatophytosis, which was treated successfully. The animal had received the usual vaccinations.

Fig. 4 Details of Fig. 3: Pseudo-stratified columnar ciliated epithelium with goblet cells in a glandular invagination (a); (histological section). Cells with kinocilia (b) and numerous goblet cells (c) as well as columnar cells (d) can also been seen. In Fig. 4d, the epithelial cell is embedded in inflammatory tissue with large and small lymphocytes and plasma cells. Near the top margin, a lymphatic cell in division can be discerned (Haemacolor stain). Both cytological and histological findings are consistent with muciparous metaplasia of the respiratory epithelium.
regularly. Apart from the purulent otitis, the cat was clinically normal. After one week of antibiotic therapy (amoxicillin-clavulanic acid; Clavaseptin®), the external ear canal could be examined with the otoscope, revealing a pink mass deep down in the ear canal. After sedation of the patient, rostrocaudal radiographs were obtained showing a soft tissue opacity in the right bulla (Fig. 6); the contralateral tympanic cavity did not show any pathological alterations. After cleaning the ear with Otoprof® (ICF, Cremona, Italy), the polyp could be visualized with the otoscope. The pharyngeal cavity was examined as described in Case 1. Due to the experience gained with the patient of Case 1, the immediate performance of ventral bulla osteotomy was suggested to the owners. In contrast to Case 1, osteotomy of the tympanic cavity and dissection of all relevant structures were performed very easily (Fig. 7). As usual, samples for bacteriological culture and cytological and histological examination were obtained during surgery. Findings showed the presence of diplococci sensitive to amoxicillin-clavulanic acid as well as haemolysing staphylococci. Over a background of inflammatory cells containing neutrophils, lymphocytes, plasma cells and macrophages, the cell-rich impression smear preparation contained ciliated epithelial cells with a relatively high cytoplasm:nucleus ratio. Histology revealed an inflammatory infiltrate covered by a layer of prismatic, ciliated epithelial cells. Both findings are consistent with inflammatory ear polyps (Figs. 3, 4 and 5). Postoperatively, reduced unilateral retractability of the tongue was examined as described in Case 1.
was noticed, which resolved within the next four weeks. The tympanic membrane took more than two months to heal completely. During the four weeks after surgery, the cat received antibiotic treatment (amoxicillin-clavulanic acid, Clavaseptin®, Vetoquinol, Vienna). 24 months after performance of the ventral bulla osteotomy, the cat was still asymptomatic.

Discussion

Histopathological and cytological findings are consistent with the changes described by Paul (2008) in human patients with middle ear disease. As the healthy mucosa of the middle ear in both men and rats is practically devoid of lymphoid follicles, an increased presence of lymphoid follicles in the feline middle ear may also be considered characteristic of chronic inflammation. In young cats, just as in children, multiple viral and bacterial infections of the upper respiratory tract may cause inflammation of this kind. In most young animals, infections of the upper respiratory tract are asymptomatic or show a mild course. As in humans, middle ear infections in the cat often occur unnoticed, as they are almost always self-limiting. The majority of cases of ear polyps published in studies refer to young animals. In only a very small number of affected animals, the condition is preceded by clinically manifest symptoms of an upper respiratory tract disease [Veir et al., 2002; Mac Phail et al., 2007]. The Maine Coon cat, in Case 1, showed nasal discharge at the time of regrowth of the polyp, which in this case was most probably a sequel to and not the cause of middle ear inflammation. In Case 2, the European Domestic Shorthair cat had suffered from massive upper respiratory tract disease at the age of eight weeks, with the ear polyp only developing seven to eight months later. In an analogy to man, living in a densely populated surroundings also represents a contributing factor in the cat (in this case, the patient lived in an animal shelter). Densely populated cat shelters carry a high risk of transmitting upper respiratory tract infections. The type of metaplasia depends to a great extent on its anatomical location. Nasopharyngeal polyps growing from the Eustachian tube into the pharynx may develop a keratinizing epithelium due to increased contact with oxygen [Esterline et al., 2005].

However, one of the authors (a pathologist) affirms that he has never diagnosed keratinization in nasopharyngeal polyps. Both cases described in this paper can be clearly related to the ear polyp only developing seven to eight months later. In an analogy to man, living in a densely populated surroundings also represents a contributing factor in the cat (in this case, the patient lived in an animal shelter). Densely populated cat shelters carry a high risk of transmitting upper respiratory tract infections.

What is striking, however, is the fact that Maine Coon cats are listed in all reported cases [Kapatkin et al., 1990; Anderson et al., 2000; Veir et al., 2002; Diel, 2008]. This may suggest a predisposing anatomical conformation of the auditory tube in this breed. Before CT and MRI had started to be widely used as imaging techniques, radiography was the only means to visualise the tympanic cavity and several authors have described this technique [Harvey et al., 2003]. However, Remedios et al. (1991) point to its low sensitivity as 25% of the findings are false negative. If the clinical presumptive diagnosis cannot be confirmed by radiology, computed tomography is the method of choice to achieve a final diagnosis.

Although the traction-avulsion method is an accepted technique for removing nasopharyngeal polyps [Werner-Tutschku, 2008], the correct initial treatment for polyps in the middle ear is still discussed controversially in the literature: While some surgeons advocate traction-avulsion of the polyp [Gotthelf, 2000; Diel, 2008], others consider ventral bulla osteotomy as the method of choice [Trevor and Martin, 1993; Anderson et al., 2000; Mullenburg and Fry, 2002; Harvey et al., 2003; Anders et al., 2008; Macphail, 2008]. Both Gotthelf (2000) and Diel (2008) removed polyps under endoscopic control by traction and avulsion applied to the stalk of the polyp. Trevor and Martin (1993), in contrast, removed ear polyps in 22 cases performing ventral bulla osteotomy; seven of these procedures were performed to remove inflammatory ear polyps and no regrowth was observed in these patients. Anderson et al. (2000) published a retrospective study with 37 cases; of these, 30 cats underwent removal of the polyp by traction-avulsion. Follow-up examinations were carried out in 22 animals, of which nine (41%) developed recurrences. That case study did not mention details of the applied technique of polyp resection. The success rate of the traction technique is clearly higher when applied to polyps in the Eustachian tube rather than to polyps in the external ear canal. However, considering the total number of surgeries for polyp removal, ventral bulla osteotomy proved to be superior to the traction method. Anders et al. (2008) found that ventral bulla osteotomy as a surgical technique can neither cure deafness caused by the polypoid condition nor damage an intact sense of hearing in an animal.

As can be deduced from literature, ventral bulla osteotomy has a high success rate as it is a reliable method to permanently remove inflammatory ear polyps without the risk of recurrences; the two cases described in this paper fully confirm this. If the traction method is performed under endoscopic control, a lower recurrence rate seems to be possible. Traction-avulsion without any visual assistance is less successful - as could be seen in Case 1. Another explanation for the reduced success rate of the traction-avulsion technique could be the fact that considerable quantities of inflammatory tissue and – even more importantly – of pus and mucus remain in the middle ear despite proper lavage. Most probably, this does not alter the microclimate in the middle ear (sero-purulent material and reduced oxygen partial pressure) sufficiently, preventing involution of the metaplasia. The situation is different with ventral bulla osteotomy. The better approach via the ventral aspect of the tympanic bulla enables performance of a meticulous curettage of the inflamed mucosa. In addition, remaining liquid and other material can easily drain and air can enter to ventilate the middle ear. The
Metaplasia of the mucosa as well as development of follicles in the tympanic cavity by infectious agents, the mucous membrane inflammation [Paul, 2008]. Independently from an invasion of the auditory tube is considered a decisive aetiological factor for middle ear dysfunction. The auditory epithelium is induced. Dysfunction of the auditory tract and frequent sneezing lead to an increased pressure in the nasal passages, and to the opening of the auditory tube. With the reflux flow from the pharynx, infectious organisms are transported into the middle ear (Sautter and Hirose, 2007).

Due to the inflammation, oedema of the epithelium in the respiratory tract and the increasing application of topical otic antibiotics for the treatment of infectious diseases of the upper respiratory tract, the auditory tube and the increasing application of topical otic preparations. Considering the state of knowledge in human medicine and the course of the two cases described in this paper together with the histological and cytological findings, the concept of a chronic otitis media with effusion as described by Sautter and Hirose (2007) may also be considered as a valid hypothesis in feline medicine.

Chronic otitis media with effusion (COME) – a possible pathophysiological course

(Fig. 8). At the beginning, there is an infection of the upper respiratory tract. This may occur without or with only mild clinical signs [Ibarolla et al., 2005]. However, a generalized lymphatic reaction of the mucous membranes in the affected region takes place. Mucosal swelling in the upper respiratory tract and frequent sneezing lead to an increased pressure in the nasal passages, and to the opening of the auditory tube. With the reflux flow from the pharynx, infectious organisms are transported into the middle ear (Sautter and Hirose, 2007).

Due to the inflammation, oedema of the epithelium in the Eustachian tube develops, resulting in an occlusion of the tube. The occluded tube produces negative pressure within the cavity system of the middle ear. With serous liquid penetrating the tympanic cavity, a process of transformation into columnar respiratory epithelium is induced. Dysfunction of the auditory tube is considered a decisive aetiological factor for middle ear inflammation [Paul, 2008]. Independently from an invasion of the tympanic cavity by infectious agents, the mucous membrane of the middle ear has already reacted. Oedema, hyperplasia and metaplasia of the mucosa as well as development of follicles in the submucosa, are the consequences [Arnold, 1977; Veir et al., 2002]. Neither mucosal-associated tissue (MALT) nor large numbers of goblet cells or intraepithelial glands form part of the healthy mucosa, but are the result of a reaction to stimuli. There is a continuous drop in oxygen partial pressure, while the partial pressure of carbon dioxide is continuously rising. Secretions and inflammatory mediators also play an important role. The result is a reduced frequency of movement of kinocilia and, as a consequence, a reduction in mucociliary clearance [Paul, 2008; Juhn et al., 2008].

The process may resolve either without treatment or due to therapeutic measures against the causative organisms and the inflammation, putting an end to the occlusion of the auditory tube and inducing a partial involution of the mucosal metaplasia. If the disease process progresses, the inflammation becomes purulent and the oxygen partial pressure decreases more and more.

The continued inflammatory process with the development of lymph follicles, increased production of mucus by the goblet cells and increased secretion of the intraepithelial glands, together with the confines of the tympanic cavity and the auditory tube activates a vicious circle [Paul, 2008], in which inflammatory mediators play an important role [Juhn et al., 2008]. Due to this self-sustaining chronification of the process, massive proliferation of the metaplastic mucosa occurs leading to the development of granulation tissue and polyps [Salvinelli et al., 1999; Paul, 2008; Juhn et al., 2008]. With increasing growth, the polyp impinges on the eardrum, which is already affected by the inflammation.

As a consequence, the tympanic membrane ruptures and bacterial contamination occurs. Toxins and/or applied drugs may damage the sense of hearing [Venker-van Haagen, 2006]. The performance of a ventral bulla osteotomy may stop the inflammatory process and even reverse it, although full recovery is not possible with this method once a sensorineural hearing loss has occurred [Anders et al., 2008]. Ventral bulla osteotomy enables surgeons to advise pet owners before surgery regarding the favourable prognosis and the possible risk of recurrence. The traction technique under optical control is a promising procedure, although a realistic evaluation of this method requires more cases as a basis for assessment. Intraoperative cytological examination showing ciliated metaplastic epithelium is a suitable tool to quickly evaluate and differentiate the tissue samples obtained during surgery. The bacterial component involved in the inflammatory process is extremely variable. In both cases described in this paper, the organisms were fully sensitive to amoxicillin-clavulanic acid. Chronic otitis media with effusion is a possible model for the pathogenesis of ear polyps in the cat.

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References


