An introduction to ear cytology in small animal patients

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Ariane Neuber DrMedVet, CertVD, DipECVD, MRCVS, discusses the process of taking samples to identify the underlying cause of otitis

Otitis is a very common problem in dogs, but is seen less frequently in cats. Clinical signs include head shaking, pruritus, erythema and visible discharge. Hyperpigmentation, lichenification and skin thickening can occur in chronic cases. Certain breeds (for example, German shepherds, spaniels, retrievers and Labradors) are recognised as being predisposed to ear disease. If there is damage to the tympanic membrane, middle or inner ear, the animal's hearing can be impaired and neurological signs can be present.

Bacterial or yeast infections are usually responsible for the visible discharge. In bacterial infections, discharges are generally yellow or green and pruritic, whereas yeast infections tend to produce a greasy, dark brownish, waxy discharge. The colour, consistency and smell of the otic discharge can give an indication of what to expect, but are no substitute for cytology.

"Otitis is usually due to an underlying cause," explains Greg Williams, veterinary technical services manager at Dechra Veterinary Products. "These causes are broadly divided into four groups: predisposing, for example conformation; primary, such as foreign bodies; secondary, for example yeasts; and perpetuating, such as otitis media. Atopic dermatitis is an example of a cause that can affect larger areas of an animal's body."

An otoscopic examination is performed to investigate otitis and check the condition of the vertical and horizontal ear canals, as well as to look at the appearance and integrity of the tympanic membrane. If only one ear appears to be involved, examine the healthy ear otoscopically first, as

there might be discharge and/or erythema deep in the horizontal canal, invisible from the outside. If both ears appear affected on examination of the pinnae, perform otoscopy on the less severely affected ear first. This should mean that the patient will experience less pain and will, therefore, be more cooperative with subsequent examination. Always use a new cone for the second ear to avoid cross contamination.

If the ear canal is too swollen and painful to allow a complete aural examination then sedation or a treatment to reduce the swelling should be considered. A full examination is required before the most appropriate treatment can be prescribed.

At this stage a cytology test can be performed to indicate infection type. Identification of an underlying cause of infection is vital, especially where the otitis is recurrent or chronic.

“Ear cytology is a quick and easy diagnosis and management tool,” Mr Williams adds. “The presence of organisms can be determined by the microscopic examination of smears and is an accurate way of targeting these organisms with appropriate treatment.”

The process

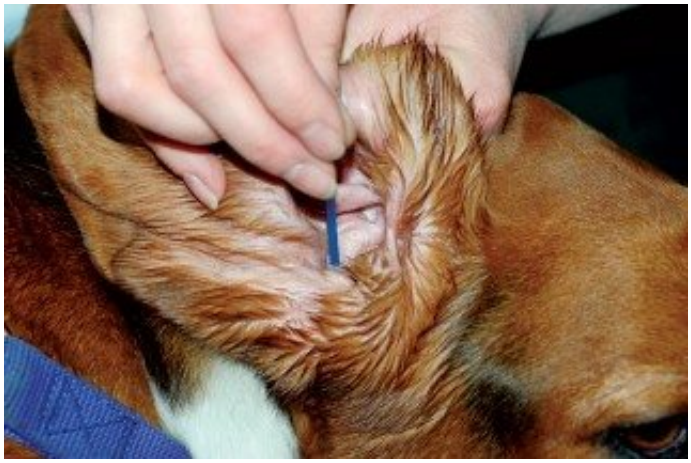

Figure 1. Carefully remove the otitic discharge with a cotton bud.

1. Getting the sample

Using a cotton bud, carefully remove the otic discharge. (Cotton buds should not be inserted into ears for any other reason than collecting samples). A second slide should be prepared and examined unstained if ear mite infestations are suspected (**Figure 1**).

2. Preparing the smear

The otic discharge should then be gently smeared on to a microscope slide by rolling the cotton bud along the glass surface to avoid disrupting the cell components. By carefully marking the slide, the material from both ears can be spread along opposite sides (**Figure 2**).

3. Fixing the preparation

The slide should then be air-dried for pruritic material or gently heat fixed (using a hair dryer) with low heat for waxy/oily samples (**Figure 3**).

4. Staining the slide

Follow the stain manufacturer's instructions. The process typically involves fixing the slide, followed by staining and counterstaining, and rinsing with water to remove excess stain (**Figure 4**). The slide can be air dried or dried gently with a hair dryer on the coolest setting. Gloves should be used to protect hands from staining.

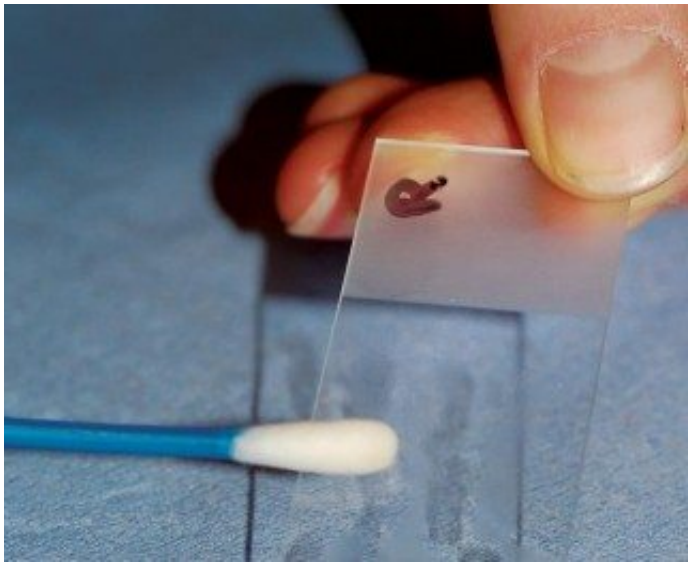

Figure 2. Otitic material from each ear can be spread along opposite sides of the slide.

Figure 3. The slide can be air dried (pruritic material) or gently heat fixed with a hair dryer (waxy/oily samples).

Figure 4. The staining process typically involves fixing the slide, staining, counterstaining and rinsing off the excess.

5. Examination under the microscope

Choose one with a good light source, binocular lenses and a mechanical moving stage to be able to choose your field to examine. Applying a drop of mineral or immersion oil over the stained material before microscopic examination, and using a cover slip, will help avoid light scatter and blurring.

Starting with the lowest magnification, choose an area that shows abundant purple staining (indicating the presence of inflammatory cells and/or organisms) then select a lens of higher magnification. Apply a further drop of immersion oil to closely examine under the oil immersion lens (x1,000) to search for cells and infectious organisms.

6. Interpretation of results

Interpretation of cytological slides comes with experience and practice. Examine the sample under high power x400 magnification to detect inflammatory cells (mainly neutrophils and macrophages) and under x1,000 magnification with oil immersion to detect cocci, rods and yeasts.

Unstained slides need to be examined for *Otodectes*under low power magnification with the condensor closed to allow for maximum contrast, and with the condenser fully open to achieve good detail during cytological examination.

7. Normal cytology

It is easier to decide what is abnormal after having examined cytology slides from a few normal ears. Occasionally, cocci and yeast in an animal with no clinical signs are within normal limits, but most fields will only contain translucent, large, polygonal cells without a nucleus, which are keratinocytes or skin cells. Some of these keratinocytes are stained purple and may be cigar shaped. When you are starting out, a good tip is to send duplicates of your slides to an experienced cytologist and compare the cytology report with your own findings.

8. Cells

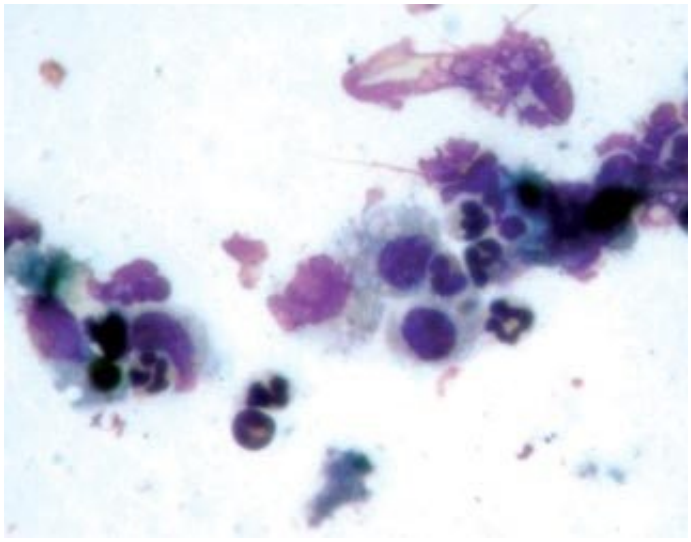

Figure 5. Neutrophils are the most commonly seen inflammatory cells in otitis cases.

You should expect to find keratinocytes on cytology, but any other cell type indicates a disease process. The most commonly seen inflammatory cells in cases of otitis are neutrophils (**Figure 5**) and macrophages. These cells usually indicate an infection, but if there are no organisms present in an ear that used to show infectious organisms and relapsed after an initial improvement, a sterile exudate might be present. This can be due to a contact allergy to the cleaner or drops used, so consider changing the preparations you are using.

In cases of pemphigus foliaceous, acantholytic cells (keratinocytes from deeper layers of the epidermis that have become detached from their neighbours) can be found. They are fairly large and round, and contain a nucleus.

9. Malassezia otitis

Malassezia pachydermatis is a very common cause of otitis externa, often detected alongside *Staphylococcus pseudintermedius.* Note the peanut (or footprint/snowman/ Russian-doll) shape of the yeast (x2,000 magnification).

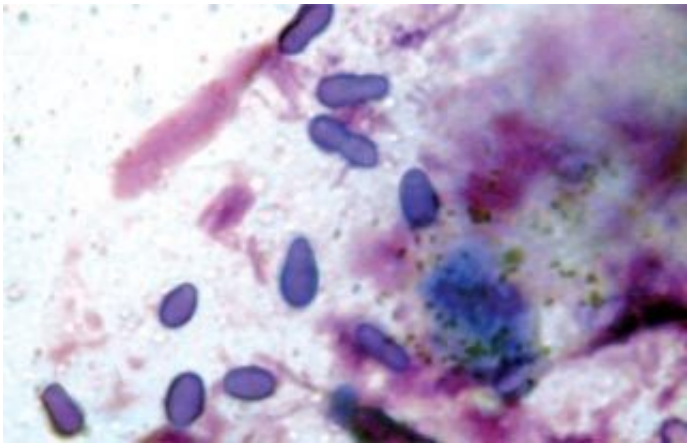

Figure 6. *Malassezia* infection – note the “peanut-shaped” yeasts.

M pachydermatis is commonly found in patients with atopic dermatitis, but also in other otitis cases (**Figure 6**). The discharge is often brown/waxy and has a characteristic yeasty odour. Although occasional yeast organisms can be found in healthy ears, treatment is advised – even when relatively small numbers of yeast cells can be seen if the clinical signs (erythema and/or pruritus) suggest they play a role in the disease. Unlike the situation with bacterial infection, *Malassezia* otitis is not often associated with an inflammatory infiltrate.

10. Coccal infection/bacterial overgrowth

Cocci will appear perfectly round, similarly sized and purple in colour (**Figure 7**). They should not be confused with melanin granules (black, brown or yellow) or irregularly shaped stain aggregates,

when focusing the microscope.

Figure 8 shows degenerate neutrophils with intra and extra-cellular cocci diagnostic for bacterial otitis, most likely associated with *S pseudintermedius.* Treatment should include thorough home cleaning if the ears are dirty, waxy or contain debris and antibiotic/corticosteroid preparations. Bacterial overgrowth with numerous cocci in the absence of any inflammatory reaction is an occasional finding.

Antibiotic therapy will improve the condition, but it is important to identify and treat the underlying cause.

11. *Pseudomonas* species otitis

Figure 9 shows numerous degenerate neutrophils and intra and extra-cellular rods. Whenever rod-shaped bacteria are seen on ear cytology, bacterial culture should be performed, because the organisms could be *P aeruginosa.*

Pseudomonas otitis requires intense medical therapy and carries a cautious prognosis. The antibiotic chosen should always be based on sensitivity testing. It is prudent to reserve some types of antibiotics, such as fluroquinolones, for conditions that have responded poorly, or are expected to respond poorly, to other classes of antibiotics.

“Ear cytology is simple to perform and requires equipment usually already present in practices, such as a microscope and slides, cotton-tipped applicators and stains. It only takes a few minutes and provides invaluable information for both initial assessment and monitoring successful progress of a treatment,” concludes Mr Williams.

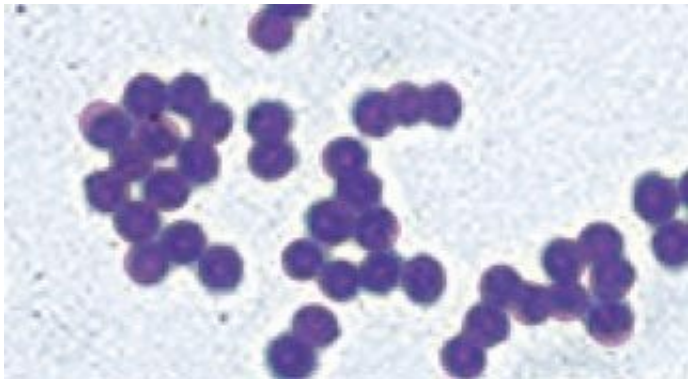

Figure 7. Cocci are perfectly round, similarly sized and are stained purple (x1,000).

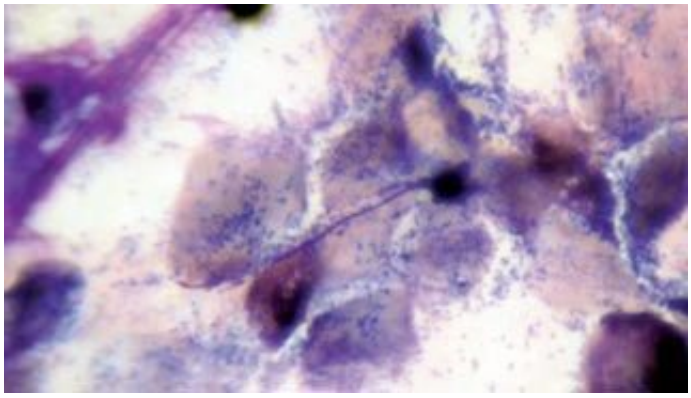

Figure 8. Bacterial overgrowth.

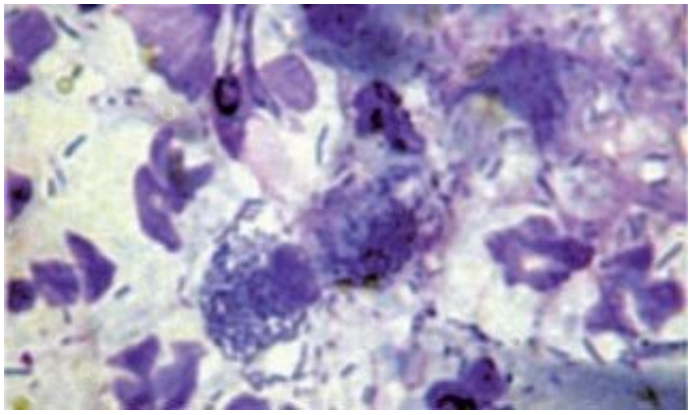

Figure 9. Degenerate neutrophils and intra and extra-cellular rods.

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