+ + + + + + CREATING CLARITY



# Mucky ears, swabs and smears: A guide to interpreting diagnostics for otitis externa

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#### **Disclosure Ariane:**

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The information contained herein is intended to provide general guidance only. As with any diagnosis or treatment you should use clinical discretion with each patient based on a complete evaluation of the patient, including history, physical exam and presentation, and laboratory data. With respect to any drug therapy or monitoring program, you should refer to a product insert, for complete description of dosage, indications, interactions, and cations, Diagnosis, treatment, and monitoring should be patient specific and is the responsibility of the veterinarian providing primary care.



II ∩c
• Review primary, secondary,
predisposing and perpetuating factors

• Develop an understanding of ear cytology, and the use of culture and microbiology results in otitis diagnosis

Identify treatment strategies for chronic otitis

#### Primary causes:

- Hypersensitivity \*
- Parasitic \*
- Space occupying lesions \*
- Foreign bodies \*
- Endocrinopathies
- Immune system pathology
- Congenital abnormalities



#### Secondary Causes:

Infections (bacterial and fungal) are secondary and represent **DYSBIOSIS** 

#### Perpetuating factors:

Chronic acquired changes that prevent resolution

- hyperplasia and thickening
- ear canal stenosis
- occlusion, fibrosis and mineralization
- otitis media
- cholesteatoma

#### Predisposing factors:

- Anatomy and conformation
- Life style and management

### **Primary causes**:

- Hypersensitivity \*
- Parasitic \*
- Space occupying lesions \*
- Foreign bodies \*
- Endocrinopathies
- Immune system pathology
- Congenital abnormalities



Drive the inflammation Allergies common! Otitis can be ONLY clinical sign Food relative common Need to identify & address to avoid relapse

Further tests needed

Overgrowth only possible due to PPP

Often cocci (Staph pseudintermedius

+/- yeast (Malassezia spp)

Other also possible

Pseudomonas particularly difficult to manage

Identify by doing cytology

When rods seen  $\rightarrow$  C&S

Biofilm!





Infections (bacterial and fungal) are secondary and represent **DYSBIOSIS** 

#### **Predisposing factors:**

- Anatomy and conformation

- Life style and management



Present prior to onset of otitis Most cannot be changed (conformation Life-style changes (swimming) may be helpful

Caused by chronic otitis

Cause self perpetuation

Anti-inflammatory meds often needed

Imaging if OM suspected



# Perpetuating factors:

Chronic acquired changes that prevent resolution

- hyperplasia and thickening
- ear canal stenosis
- occlusion, fibrosis and mineralization
- otitis media
- cholesteatoma

# Are you looking at the ears?



### Ear anatomy

- Explain to owners
- Epithelial migration  $\rightarrow$  self cleaning process
  - Gets overwhelmed in a diseased ear



# examination is essential in otitis externa

- Evaluate for primary, predisposing and perpetuating factors;
- Evaluate the amount and type of exudate in the ear canals;
- Estimate the amount inflammation;
- Identify hyperplasia, masses, and foreign bodies;
- Determine the status of the tympanic membrane
- Get clues for S



# Ear exam: palpate, look, smell, look deeper...

- Evaluate for stenosis, hardening of ear canal
- Evaluate for other signs of generalized & dermatological

#### disease

- Get clues for possible Ps
- Get help formulating therapy (nature of otic discharge, status of TM)

## Ear cytology

- Provides information that can help guide treatment
- Most important test!
- Quick
- Inexpensive
- EVERY otitis patient & BOTH ears: initial consult & follow up
- Sometimes allows identification of primary causes (e.g. parasitic) or rule out ceruminous otitis only
- Most will have variable numbers of :
  - Keratinaceous material
  - Yeast or bacterial organisms
  - Variable inflammation
- Quantification and monitoring of response to treatment



### How?

#### **Cotton bud/gloves**

#### Role out/dab on





### Sample processing

#### DiffQuick



#### How to...

- Fixation: heat? Solution 1?
- Air dry
- Eosin red?
- Methylen blue!!!

### Different ears – different findings

Left ear – Malassezia only

**Right ear – Malassezia and cocci** 



### Start ASAP and often – it gets easier



# • Start with low power

- Examine the whole smear with a "battle ship movement"
- Choose the most cellular / well spread/ well preserved areas
- Increase to higher power field for identification and quantification of organisms:
- Look for organisms: cocci, rods, yeast
- Look for inflammatory cells





## Ceruminous Otitis

No/few microorganisms detected

- No parasites
- No/few bacteria
- No/few yeasts

Idiopathic seborrhoea and other keratinization disorders

The excess cerumen will predispose to bacterial and Malassezia overgrowth



### Otodectes cynotis

Parasites are primary cause of otitis

May have secondary bacterial overgrowth/infection

Best diagnosed in fresh smears

Up to 85% of feline otitis externa

Reinfestation can occur after treatment (specially from other pets)



#### Site:

#### LEFT EAR :

Aerobic Culture - Ear

#### Isolate 1

Profuse growth:Staphylococcus felis

Antibiotic	Result	MIC	Sensiti	vity Range	
Ampicillin (1)	Resistant				
Amoxicillin (1)	Resistant				
Amoxicillin-Clavulanic acid (1)	SENSITIVE				
Cloxacillin (1)	SENSITIVE				
Enrofloxacin (2)	SENSITIVE	<=0.5	0.5	Siir	4
Marbofloxacin (2)	SENSITIVE	<=0.5	0.5	Ssirr	8
Pradofloxacin (2)	SENSITIVE	<=0.12	0.12	Ssiirr	4
Neomycin (2)	SENSITIVE	<=2	2	Sssir	32
Chloramphenicol (1)	SENSITIVE	<=4	4	Ssirr	64
Ofloxacin (2)	SENSITIVE				
Fusidic acid (2)	SENSITIVE	<=0.5	0.5	Ssmm	32
Minocycline (1)	SENSITIVE	<=0.5	0.5	Ssssir	16
Cephalexin (1)	SENSITIVE				
Gentamicin (2)	SENSITIVE	<=0.5	0.5	Ssssir	16
Ciprofloxacin (2)	SENSITIVE				
Florfenicol (1)	SENSITIVE	<=4	4	Ssrr	32

Organism identified by MALDI-TOF as Staphylococcus felis (coagulase-negative), which is regarded as a primary pathogen when recovered from urine. It is potentially pathogenic when recovered from skin, wounds, ears, abscess or conjunctiva.

#### Note:

Standardised susceptibility tests do not reflect in vivo activity of topical antibiotics due to the high levels achieved in the target site with topical administration. Generic antibiotics quoted. The choice of antibiotic and knowledge of any contraindications is the Veterinary Surgeons responsibility. MIC units expressed in ug/ml. Antibiotics without a MIC have been predicted using international guidelines. For more information on interpretation of MICs visit idexx.co.uk/MIC



### Neutrophils only

- Inflammation is not infection!
- Remember allergies and foreign bodies!





## The ear is not sterile...

- Just because we see/grow bacteria does not implicate bacterial otitis

- Consider **DYSBIOSIS** 

- High numbers of organisms overgrowth and/or infection
- Phagocytosis and intracellular bacteria ongoing active infection
- If reporting number of organisms present:
   average of 10 oil immersion (x1000) microscopy fields:

DEBATABLE!

- use semiquantitative assessment



# Common organisms found in normal ears and in otitis

#### **Normal ears**

- Malassezia pachydermatis\*, other Malassezia spp. and other yeasts
- Staphylococcus pseudintermedius\*
- Staphylococcus schleiferi subsp. coagulans
- Coagulase-negative staphylococci
- Corynebacterium spp. +
- Streptococcus spp.
- Other species
  - Actinobacteria, Proteobacteria, Firmicutes, and Bacteroidetes

#### Ears with otitis externa

- Malassezia spp.\*
- Staphylococcus pseudintermedius\* and other staphylococci
- Pseudomonas aeruginosa\*
- Proteus mirabilis\*
- Beta-haemolytic streptococci (e.g. S. canis)
- Corynebacterium spp.
- Enterococcus spp.
- Escherichia coli



Otitis media: Also consider anaerobes



## **Bacterial Otitis**





#### Septic Neutrophilic Inflammation Bacterial Otitis

#### Biofilm can complicate the picture



#### Biofilm and Bacilli consider Pseudomonas

CODIO GUILLIO LU

Isolate 1

Profuse growth:Pseudomonas aeruginosa

Antibiotic	Result	MIC	Sensit	vity Range	
Ampicillin (1)	Resistant	N/A		(Intrinsic R)	
Amoxicillin-Clavulanic acid (1)	Resistant	N/A		(Intrinsic R)	
Enrofloxacin (2)	SENSITIVE	0.5	0.12	ssSiir	4
Gentamicin (2)	SENSITIVE	<=1	1	Sssir	16
Clindamycin (1)	Resistant	N/A		(Intrinsic R)	
Amikacin (2)	SENSITIVE	<=2	2	Ssssir	64
Tobramycin (2)	SENSITIVE				
Polymyxin B (3)	Intermediate	1	0.25	iilirrr	16
Cephalexin (1)	Resistant	N/A		(Intrinsic R)	
Marbofloxacin (2)	SENSITIVE	1	0.5	sSir	4
Cefovecin (2)	Resistant	N/A	0.070	(Intrinsic R)	2.5.

Pseudomonas aeruginosa may develop resistance during prolonged therapy with all antimicrobial agents. Therefore, isolates that are initially susceptible may become resistant within three or four days after initiation of therapy. Testing of repeat isolates may be warranted.

in mixed bacterial growth.

#### Note:

Standardised susceptibility tests do not reflect in vivo activity of topical antibiotics due to the high levels achieved in the target site with topical administration. Generic antibiotics quoted. The choice of antibiotic and knowledge of any contraindications is the Veterinary Surgeons responsibility. MIC units expressed in ug/ml. Antibiotics without a MIC have been predicted using international guidelines. For more information on interpretation of MICs visit idexx.co.uk/MIC





### Malasetias

- Varies with studies
- dry hpf (40x)
- mean yeasts/hpf  $\geq 1 5 10$  abnormal

However:

overlap in yeast densities in skin samples from healthy and diseased dogs
 relatively small numbers of organisms may lead to skin disease in sensitised individuals

"Factors such as important variations in anatomical site, breed, sampling method and host immune status commonly thwart the interpretation of the clinical significance of an observed population ("XX yeasts in YY fields"); trial therapy is routinely required to establish this."

Bond R, et al Biology, diagnosis and treatment of Malassezia dermatitis in dogs and cats: Clinical Consensus Guidelines of the World Association for Veterinary Dermatology. Vet Dermatol. 2020 Feb;31(1):75. doi: 10.1111/vde.12834. PMID: 31957203.

#### Is Culture Beneficial?

- May have limited benefit in otitis externa
- Allows to identify if only mixed flora
- Most cases of infection are due to :
  - Malassezia spp
  - Staphylococus spp (cocci)
  - Pseudomonas (bacilli)
- More useful in recurrent cases or with organisms with unusual morphology
  - e.g., coryneform, cocci-bacilli, filaments, yeasts, hyphae, etc
- Direct microscopy findings aid in the determination of clinical significance of isolates e.g., bacterial morphology associated with inflammation and phagocytosis.
  - Always do Cytology before and when doing culture



#### Is Culture Beneficial?

IDEXX SERVICES: CANA, EARSW SAMPLES RECEIVED: Pink cap e-swab

#### MICROBIOLOGY

\*Anaerobic Culture a

\*Site:

Aerobic Culture - Ear

Isolate 1

Profuse growth:Pseudomonas aeruginosa

Moderate growth mixed anaerobes

BOTH EARS :

Antibiotic	Result	MIC	Sensitivity Range			
*Amikacin	SENSITIVE	<=2	2	Ssssir	64	
*Gentamicin	SENSITIVE	<=1	1	Sssir	16	
*Ciprofloxacin	SENSITIVE	0.25	0.06	ssSssir	4	
*Enrofloxacin	Intermediate	1	0.12	ssslir	4	
*Marbofloxacin	SENSITIVE	<=0.5	0.5	Ssir	4	
*Polymixin B	SENSITIVE	1	0.25	ssSsrrr	16	
*Ofloxacin	SENSITIVE					

in mixed bacterial growth.

- ASTs are based on systemic breakpoints
- The results can be poorly predictive of the response to topical treatment.
  - If R on AST may respond in vivo due to high concentration that can be achieved on site
  - If S on AST may not respond in vivo due to local factors (e.g. inflammation, biofilm, ear stenosis, etc)
- May be useful with bacilli infection (e.g. Pseudomonas vs Enterobacterales vs Corynebacteria)



#### Is Culture Beneficial?

Typical indications for Culture include the following:

- Chronic otitis associated with bacteria (cocci and/or rods) seen on cytology
- Rods (bacilli) seen on cytology
- Organisms with unusual morphology
- Suspected or confirmed cases of otitis media (systemic therapy may be indicated)
- History of multidrug-resistant bacteria
- History of long-term oral or topical antibiotic therapy (including for other conditions
- Bacteria persisting on cytology despite apparently appropriate therapy



### Culture Beneficial?

SPECIES	AGE 9y 9m (03/06/2014)			
Canine				
BREED	SEX	NEUTERED	ENTIRE	
Basset Hound	F	x		



DOG HAS BEEN ON GENTAMICIN TOPICALLY FOR 4-6 WEEKS,



Ear Aerobic Culture

Profuse growth:Pseudomo	nas aeruginosa		
Antibiotic	Result	MIC	Sensitivity Range
Ampicillin (1)	Resistant	 N / Δ	(Intrinsic R)
Amoxicillin-Clavulanic	acid (1)Resistant	N/A	(Intrinsic R)
Enrofloxacin (2)	Intermediate	1	0.12 sssIir 4
Gentamicin (2)	Resistant	>=16	1 sssiR 16
Clindamycin (1)	Resistant	N/A	(Intrinsic R)
Amikacin (2)	Resistant	>=64	2 ssssiR 64
Tobramycin (2)	Resistant		
Polymyxin B (3)	Intermediate	1	0.25 iiIirrr 16
Ofloxacin (2)	SENSITIVE		
Cephalexin (1)	Resistant	N/A	(Intrinsic R)
Marbofloxacin (2)	SENSITIVE	1	0.5 sSir 4
Cefovecin (2)	Resistant	N/A	(Intrinsic R)
Ciprofloxacin (2)	SENSITIVE	0.5	0.06 sssSirr 4

Pseudomonas aeruginosa may develop resistance during prolonged therapy with all antimicrobial agents. Therefore, isolates that are initially susceptible may become resistant within three or four days after initiation of therapy. Testing of repeat isolates may be warranted.

Standardised susceptibility tests do not reflect in vivo activity of topical antibiotics due to the high levels achieved in the target site with topical administration. Please note that topical treatment with the antimicrobials listed as intermediate (e.g. Polymyxin B) may be effective in this case given the high concentrations achieved at the site.



## Take Home MSG

- Physical exam and looking for primary causes essential
- Cytology will provide essential information on initial consult...
- ...and at every subsequent visit until cure is achieved
- Recurrent cases need addressing underlying causes
- Culture may be useful in identifying which organisms are present
  - Overgrowth of normal flora
  - Dysbiosis
  - Infection
  - AST provides limited but valuable information

## Traditional ear cytology can be challenging



Photo courtesy of Dr. Elizabeth Layne

### IDEXX inVue Dx<sup>™</sup> analyzer: ear cytology workflow









### IDEXX inVue Dx<sup>™</sup> analyzer: Ear cytology



IDEXX VetLab Statio	n		Q	< <u></u>	\$	9:30
SADIE 123456 Canine   Poodle	e   Female   4	y   Profile ∽		Back	A	\dd Te
Result Details			• •	Tran	isfer Re	esults
✓ Cytology	1/10/24 8:02 AM					
Left Ear Bacteria, Cocci	3-4+	Numerous coccoid-shaped bacteria present				
Bacteria, Rods	0-1+	Consistent with normal flora				
'east	0-1+	Consistent with normal flora				
VBC	Present					
lites	Absent					
Diagnostic Considerations	Bacterial otitis the presence o Consider unde intensive diagr foreign body p	with coccoid-shaped bacteria. The finding of numerous coccoid- of bacterial otitis. rrlying causes of otitis externa, Typically these patients require lo nostics/therapies (otic irrigation, advanced imaging to investigate resence).	shaped bacteria is 95 nger duration of trea potential for tumor (	% specific f tment or m or otitis me	or ore dia,	
mages						
Right Ear						
Bacteria, Cocci	3-4+	Numerous coccoid-shaped bacteria present				
Bacteria, Rods	0-1+	Consistent with normal flora				
'east	0-1+	Numerous yeast present				
VBC	Present					
Mites	Absent					
Diagnostic Considerations	Bacterial otitis the presence o Consider unde more intensive media, foreign	with coccoid-shaped bacteria. The finding of numerous coccoid- f bacterial otitis. rlying causes of otitis externa, Typically these patients require lo e diagnostics/therapies (otic irrigation, advanced imaging to inves body presence).	shaped bacteria is 95 nger duration of trea tigate potential for tu	% specific f tment or umor or otit	or	
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## References

- Shaw, S. (2016). Pathogens in otitis externa: Diagnostic techniques to identify secondary causes of ear disease. In Practice, 38. <u>https://doi.org/10.1136/inp.i461</u>
- Hoffmann AR, Patterson AP, Diesel A, et al. The skin microbiome in healthy and allergic dogs. PLoS One. 2014;9(1):e83197. doi: 10.1371/journal.pone.0083197
- Apostolopoulos N, Glaeser SP, Bagwe R, et al. Description and comparison of the skin and ear canal microbiota of non-allergic and allergic German shepherd dogs using next generation sequencing. PLoS One. 2021;16(5):e0250695. doi:10.1371/journal.pone.0250695
- Ngo J, Taminiau B, Fall PA, Daube G, Fontaine J. Ear canal microbiota—a comparison between healthy dogs and atopic dogs without clinical signs of otitis externa. Vet Dermatol. 2018;29(5):425-e140. doi:10.1111/vde.12674
- August JR. Otitis externa: A disease of multifactorial etiology. Vet Clin North Am Small Anim Pract. 1988;18(4):731–742. doi:10.1016/S0195-5616(88)50076-1
- Murphy KM. A review of techniques for the investigation of otitis externa and otitis media. Clin Tech Small Anim Pract. 2001;16(4):236–241. doi:10.1053/svms.2001.27601
- O'Neill DG, Volk AV, Soares T, Church DB, Brodbelt DC, Pegram C. Frequency and predisposing factors for canine otitis externa in the UK—a primary veterinary care epidemiological view. Canine Med Genet. 2021;8(1):7. doi:10.1186/s40575-021-00106-1
- Angus JC. Otic cytology in health and disease. Vet Clin North Am Small Anim Pract. 2004 Mar;34(2):411-24. doi: 10.1016/j.cvsm.2003.10.005. PMID: 15062616.
- Nuttall T. Managing recurrent otitis externa in dogs: what have we learned and what can we do better? J Am Vet Med Assoc. 2023 Apr 7;261(S1):S10-S22. doi: 10.2460/javma.23.01.0002. PMID: 37019436.
- Tang S, Prem A, Tjokrosurjo J, et al. The canine skin and ear microbiome: a comprehensive survey of pathogens implicated in canine skin and ear infections using a novel next-generation-sequencing-based assay. Vet Microbiol. 2020;247:108764. doi:10.1016/j.vetmic.2020.108764
- Bond R, Morris DO, Guillot J, Bensignor EJ, Robson D, Mason KV, Kano R, Hill PB. Biology, diagnosis and treatment of Malassezia dermatitis in dogs and cats: Clinical Consensus Guidelines of the World Association for Veterinary Dermatology. Vet Dermatol. 2020 Feb;31(1):75. doi: 10.1111/vde.12834. PMID: 31957203.