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# Otoscopy and aural cytological findings in a population of rescue cats and cases in a referral small animal hospital in England and Wales

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**Abstract**

**Objectives**

Otitis externa is seen clinically in cats although studies investigating this within the UK are lacking. The objective of this study was to investigate the prevalence of *Otodectes cynotis* mites and microbial infection in the ear canals of cats in various rescue / charitable centres and a referral hospital.

**Methods**

Otoscopy was performed in 332 cats from a range of sources. Otoscopic findings were noted, including the gross visualisation of *Otodectes*. A sample of cerumen was collected for cytological evaluation and a cerumen smear for detection of *Otodectes* mites if there was a large amount of black or brown aural exudate on otoscopy ~~sufficient exudate~~ for a smear to be mounted in paraffin oil.

**Results**

~~Otoscopic evidence of~~ *Otodectes cynotis* infestation was noted in 3 / 341 cats (0.9 %, 95 % CI = 0.3 - 2.6 %). A total of 129 / 341 (37.8 % CI = 32.7 – 43.0%) cats were found to have *Malassezia* species within one or both ears. Bacteria were found unilaterally in 9 / 341 (2.6 % CI = 1.4 – 4.9 %) cats. Analysis of the cytological findings showed an increased likelihood for *Malassezia* to be present ~~in older cats~~ as age increased (Pearson r = 0.204, P < 0.001, n=293). There was also an increased likelihood of finding *Malassezia* in both ears if found within one ear (r = 0.499, P < 0.001, n = 327). There was a positive correlation between the number of *Malassezia* organisms and the quantity of aural exudate (r = 0.778, P < 0.001, n = 338). Only 10 / 332 cats were found to have no exudate at all upon otoscopy. Cats where *Otodectes* infestation were noted (n = 3), had moderate or large quantities of cerumen. All cats with bacteria on cytology were found to have small to large quantities of aural exudate present.

**Conclusions and relevance**

This study shows that there was a low prevalence of *O. cynotis* in this cohort of cats in the United Kingdom. In normal cats it was not unusual to find *Malassezia* microorganisms upon aural cytology, bacteria were noted far less frequently and in two cats this was associated with underlying anatomical pathology.

## Introduction

Otitis externa is seen more frequently in dogs than cats.[1-3] Many studies have investigated the prevalence of otitis externa in cats, although studies in the United Kingdom are lacking.

*Malassezia spp.* are known to be part of the normal aural microflora in cats.[4-7] Many studies have used ear swabs for bacterial and fungal culture to investigate the aural microflora of cats, with and without otitis externa, but fewer studies have used cytology for investigating the normal feline aural microflora. *Malassezia* yeasts were cultured from 95.1% and 48.4% of cats in Iran with and without otitis externa, respectively.[8] In a study performed in Brazil, *Malassezia spp.* were isolated (also using fungal culture) in 75 % and 28 % of cats with and without otitis externa, respectively.[9] ~~Many studies have taken ear swabs for bacterial and fungal culture to investigate the aural microflora of cats, with and without otitis externa, but fewer studies have used cytology for investigating the normal feline aural microflora~~. A study performed in Belgium examined a stray population and reported 74 % of cats to have *Malassezia spp.* in one or both ears based upon cytological examination alone.[10] Fifty-five per cent of cats were found to have *Malassezia* upon aural cytological examination and *Otodectes cynotis* were found in 29.4 % of cats in an Italian study also examining stray cats.[11] In a study performed in France investigating pet cats, fifteen healthy cats were examined and no *Malassezia* yeasts were detected, bacteria were isolated from a single ear.[12] In a study performed in the USA, fifty-two privately owned cats were examined using aural cytology, yeasts were detected in 83 %, and coccoid shaped bacteria in 71 % of cats.[6] The median number of microorganisms per high power dry field was 0.2 and 0.3 for *Malassezia* and coccoid shaped bacteria respectively. Far higher numbers of *Malassezia* and bacteria were found in a study performed in Spain, where sixteen normal cats were examined; more than or equal to 12 *Malassezia* and more than or equal to 15 bacteria per high power dry field were found.[5]

There is a marked variation in the reported prevalence of *O. cynotis* in cats, ranging from 0.9 % in Australia[13] to 83.7 % in the United Kingdom.[14] Many of these studies have examined cats from a feral population which may not be representative of the population seen in primary veterinary care or referral practice. A study from the UK published in 1955 examined 153 cats at post-mortem and the incidence of *O. cynotis* was reported to be 51 %.[15]

The aims of this study were to examine the external ear canal otoscopically and evaluate cytological findings in a large population of cats in a non-feral environment from rescue centres, and in cats presenting to a referral Small Animal Hospital and first opinion practice, from centres in England and Wales.

## Materials and Methods

Sampling and data collection

Three hundred and forty-one cats were included in this study. Ethical approval was obtained. Cats were recruited from across six rescue centres in the South West of England and South Wales, London and Birmingham (total n= 288, range per centre = 13 to 82). Cats were also recruited from Langford Small Animal Practice and Small Animal Referral Hospital (n=53). Owners of the rescue centres and pet cats gave written or verbal telephone consent for cats to be enrolled on the study. The centre, age, sex, reproductive status, reason for examination, ~~if~~ whether there were 'in contact' animals, use of ectoparasite control and frequency, lifestyle (indoor / outdoor) and concurrent medication were recorded for each cat. If treatment was recommended based upon the aural and cytological findings, this was also noted.

Cytological and microscopic evaluation

A clean, non-sterile cotton bud was inserted and rotated into the vertical ear canal to obtain a sample of cerumen for cytological examination. The same person collected the sample and characterised the colour of the cerumen. The sample was rolled onto a clean microscope slide in two lines to distribute the exudate evenly over the slide. The microscope slide was stained with a modified Wright's stain (Diff-Quik®; Atom Scientific, Manchester, UK), with five one second dips in each of the component three solutions and then the slides were washed and allowed to air dry.

If there was a sufficient quantity of aural exudate present consistent with that described in *O. cynotis* infected cats,[15,16] an extra sample was taken and mounted in paraffin oil on a microscopy slide and a cover slip was applied. This was examined under a low power using x 40 or x 100 magnification and the presence of *Otodectes* or *Demodex* adult mites, or their immature life cycle stages (eggs, larvae and nymphs) was noted.

Each stained microscope slide was examined by the same operator using the same microscope (Olympus, Southend-on-sea, UK), blinded to the previously noted otoscopy findings. Ten fields were examined using immersion oil. Each slide had the total number of *Malassezia* recorded (the sum of all ten fields) and the average number per oil immersion field (OIF) was calculated.

The number of bacteria were classified using a previously reported method,[17] shown in table 1.

| Classification | Description |
|---|---|
| 0 | No bacteria / yeast / inflammatory cells |
| 1+ | Occasional bacteria / yeast / inflammatory cells present, but slide must be scanned carefully for detection |
| 2+ | Bacteria/ yeast / inflammatory cells present in low numbers, but detectable rapidly without difficulties |
| 3+ | Bacteria / yeast / inflammatory cells present in larger numbers and detectable rapidly without any difficulties |
| 4+ | Massive amounts of bacteria / yeast / inflammatory cells present and detectable rapidly without difficulties |

Table 1 Classification of the quantitative scale used to assess bacteria (based on a previous study[17])

Inflammatory cells, saprophytes, squamous cells and melanin granules were noted as being present or absent for the whole of the slide.

If otitis (defined as aural discomfort, erythema or abnormal exudate) was noted upon otoscopy whilst examining a cat, cytology samples were evaluated ~~performed~~ on the same day so that medication could be prescribed.

Otoscopy

Each external ear canal was examined using a Heine veterinary hand held otoscope (HEINE Optotechnik, Herrsching, Germany) with a small otoscope head if cerumen sampling was well tolerated. A small number of cats were examined under sedation or general anaesthetic if they were undergoing a procedure at Langford Small Animal Hospital or Small Animal Practice. Table 2 shows the scale used for otoscopic assessment which is an adaptation of a previously reported method of aural clinical scoring.[18] The presence of a space occupying lesion such as a polyp or mass, was noted. Assessment also included the gross presence of *Otodectes* mites (yes / no) and whether it was possible to visualise the tympanic membrane (yes / no). Any other dermatological lesions (ears or whole skin) were noted.

Data were entered into an Excel (Microsoft) spreadsheet and statistical tests were performed using IBM SPSS Statistics v24 (SPSS, Armonk, NY, USA). Overall prevalences are reported as a percentage of cats, together with a 95 % confidence interval of the estimate calculated using Wilson's method.

| Grade | Quantity of cerumen | Degree of ulceration | Erythema |
|---|---|---|---|
| 0 | None | None | None |
| 1 | Small | Mild | Mild |
| 2 | Moderate | Moderate | Moderate |
| 3 | Large | Severe | Severe |

Table 2 Clinical parameters and scoring system

**Results**

*Population*

Three hundred and forty-one cats were included in this study aged from three weeks ~~to~~ and eighteen years. Two hundred and ninety-one cats were reported to have had contact with other cats or dogs. Two hundred and seventy-five cats had an indoor / outdoor lifestyle, 45 cats were indoor only, one cat was outdoor only and for 20 cats their lifestyle was unknown.

One hundred and forty (41.1 %), cats were male and 198 (58.1 %) were female. One hundred and fifteen (33.7 %) were entire, 224 (65.7 %) cats were neutered with missing data for two cats. Twenty-seven (7.9 %) cats were receiving systemic therapy or topical ear medication at the time of sampling. Fifteen different breeds were sampled (see table S1 in Supplementary material), however, the majority (94.7 %) were classified as domestic long, medium or shorthair, with other breed classifications poorly represented.

Eight out of 341 (2.3 %) cats, were noted to have focal to generalised signs of dermatological disease including moist and crusting dermatitis, abscessation, pinnal comedones, hypotrichosis of the ventrum, miliary dermatitis, chin acne, pododermatitis, paronychia, ~~and~~ over grooming (see figure 1)~~.~~ and exfoliative dermatitis (see figure 4 in Supplementary material).

Figure 1 Concurrent dermatological disease found in some cats
a) erythema of the muzzle and chin along with mild feline acne, b) moderate feline acne over the intermandibular region, c) ceruminous cystomatosis

*Otoscopic examination*

Otoscopy was generally well tolerated although it was not possible in ~~10~~ 9 / 341 ~~(2.9 %)~~ (2.6 %) cats in either one or both ears. The tympanic membrane was visualised partially or completely in 306 / 332 (91.2%) cats in one or both ears. Three cats (0.9%, CI = 0.3-2.5%) were found to have *O. cynotis* adult mites visible upon otoscopy within one or both ears (confirmed using microscopy).

*Cerumen smear and cytological examination findings*

An extra sample of aural exudate for low power microscopy (40 x and 100 x) was taken in 13 cats (3.8 %), eleven of these cats had excessive aural exudate bilaterally, two had unilateral presentation, therefore twenty-four exudate samples mounted in paraffin oil were examined for microscopic evidence of mites. Cytological findings are shown in table 3. *Demodex gatoi* was noted unilaterally in one cat. *Otodectes cynotis* was noted in 3 / 341 (0.9%, CI = 0.3-2.5%) cats using microscopy (see figure S1 in Supplementary material). Two of the three cats had bilateral *O. cynotis* infestation. One cat with bilateral infestation microscopically only had gross otoscopic evidence in one ear.

| Cytological findings | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Malassezia* | Coccoid shaped bacteria | Rod shaped bacteria | Coccoid and rod shaped bacteria | *Otodectes cynotis* | *Demodex gatoi* | Melanin granules | Saprophytes |
| Number of cats with cytological findings (out of 341 cats) | 62 (bilateral)<br>67 (unilateral) | 7 (unilateral) | 1 (unilateral) | 1 (unilateral) | 2 (bilateral)<br>1 (unilateral) | 1 (unilateral) | 212 (bilateral)<br>85 (unilateral) | 311(bilateral)<br>26 (unilateral) |

Table 3 Cytological findings

Table S2 in Supplementary material shows the otoscopic and cytological findings of four cats with evidence of *Otodectes* and / or *Demodex.* Neither bacteria or inflammatory cells were noted.

Some of the cytological findings that were noted are shown in figure S2 of the Supplementary material.

~~Sixty-two out of 341 cats (18.1 %) were found to have *Malassezia* bilaterally; sixty-seven cats had *Malassezia* unilaterally (19.5 %).~~ There was an increased likelihood for *Malassezia* to be present with increasing age ~~as age increases~~ ~~in older cats~~ (Pearson r = 0.204, $P < 0.001$, n = 293) and an increased likelihood of finding *Malassezia* in both ears if found within one ear (r = 0.499, $P = <0.001$, n = 327)**.** There was a significant correlation between the number of *Malassezia* and the quantity of aural exudate (r = 0.778, $P < 0.001$, n = 338).

Thirty-nine cats were found to have otitis externa based on either having presented for otitis, or incidental findings upon otoscopy (aural discomfort, erythema, abnormal exudate, presence of a mass or *O. cynotis*) or *O. cynotis* visible microscopically. ~~(e.g. erythema or the presence of *O. cynotis*)~~ ~~and examination of the ear pinnae~~ ~~or cerumen microscopy~~. Four cats presented to the dermatology service at Langford Small Animal Hospital with otitis as a presenting complaint, in thirty-five cats it was an incidental finding. A two-sided, exact Mann Whitney test showed there to be a significant difference in the number of *Malassezia* per OIF between the two groups; the mean number for the otitis group was 0.687 (CI = 0.153 to 1.380) compared with 0.169 (CI = 0.114 to 0.228) in the group of cats without clinical signs of otitis. ~~detectable clinically.~~

Those cats with otitis are shown in table ~~3~~ 4 with the underlying aetiology of the otitis (if known).

| | *Demodex gatoi* | *Otodectes cynotis* | Aural mass / polyp | Allergic skin disease | Ceruminous cystomatosis | Generalised skin disease | Unknown |
|---|---|---|---|---|---|---|---|
| Number of cats | 1 | 3 | 3 | 4 | 2 | 2 | 24 |

Table ~~3~~ 4 Cats with otitis and the underlying aetiology

~~Nine (2.6 %) cats were found to have environmental contaminants (saprophytes) on ear cytology.~~

Bacteria were found unilaterally in 9 / 341 (2.6 %) cats. Six of these cats were in the non-otitis group and three were from the otitis group. Seven of these cats had coccoid shaped bacteria only, one cat had both rod and coccoid shaped bacteria and one cat had rod shaped bacteria only. Those cats with higher ~~larger~~ numbers of bacteria (3 or 4+) were within the otitis group. Two of these cats (one with rod shaped bacteria) were found to have a space occupying lesion documented using computed tomography, within the ear where bacterial infection was found. Table 3 in Supplementary material shows the otoscopic and cytological findings of cats where bacteria were found upon cytology. Mites were not detected in any of these cats.

~~Melanin granules were noted bilaterally in 212 / 341 (62.2 %) cats, and unilaterally in 85 (24.9 %) cats. Squamous cells were noted bilaterally in 311 / 341 (91.2 %) cats and unilaterally in 26 (7.6 %) cats.~~

Some form of ectoparasite control had been used in 278 / 341 (81.3 %) of cats at the time of enrolment into the study. ~~Nineteen (5.6 %)~~ Thirty eight / 341 (11.14 %) of these cats received regular ectoparasite control at the manufacturers recommended frequency of application.

## Discussion

The primary aims of this study were to investigate both the prevalence of *O. cynotis* in a large cohort of cats and to examine the ear cytology of clinically normal cats from both a rescue centre and veterinary practice setting within the UK. ~~and to examine the ear cytology of clinically normal cats.~~ Those cats presenting for otitis or with disease noted incidentally, were removed when analysing the data for normal ear cytology values. To the best of the authors' knowledge, there have not been any recent studies investigating the prevalence of *O. cynotis*

within a large cohort of cats in the UK, and there have been only three studies that have evaluated the normal external ear cytology in cats.[5,6,12]

This study found that the prevalence of *O. cynotis* was low, recorded as 0.9 %. This result is in agreement with a Belgian study (2%),[10] an Australian study (<0.1%)[13] and a Portuguese study (2.2%).[19] Far higher numbers were reported in a Greek study (25.5%),[20] Italian study (29.4%)[11] and in a study from the United States (37%)~~,~~.[21] Climate differences between countries could also account for these differences. A far older study from 1955 in the UK showed that the prevalence was 55%,[15] although this is during a time period where preventative acaricidal products were not available and therefore may have influenced the findings in the population studied.

The prevalence may have been underestimated in this study as low power microscopy was only performed in samples from those cats with a large amount of black or brown aural exudate on otoscopy. In a previous study,[21] otoscopic examination was normal in eight cats that were positive microscopically (in total seventy-four out of two hundred cats were found to have *Otodectes* microscopically) which suggests that all ears should have a cerumen sample taken for paraffin oil microscopy, even if otoscopy does not reveal a large amount of the classical brown / black exudate seen in Otodectic mange.[15] In one study,[20] the ear canal was flushed with 1-2 ml of mineral oil along with vigorous massaging to determine the presence of *O. cynotis* as there was concern that the cotton swab technique was less efficient than flushing. Anecdotally, the risk of ototoxicity and discomfort to cats with this method was deemed unacceptable for use in our study. An alternative method of detecting *O. cynotis* infection is the use of PCR[22] which could be evaluated in future studies. This may however be cost prohibitive in clinical practice and therefore trial treatment may be elected in the first instance. The life ~~style~~ cycle stage of the *O. cynotis* mite seen upon microscopy was not noted in this study.

Another reason for a low prevalence in this study compared with investigations on stray populations could also be attributable to owned and rescue cats receiving ectoparasite control (many of which have acaricidal activity), albeit not necessarily at the manufacturer's recommended application frequency. Most rescue centres tend to apply ectoparasite control routinely when cats are admitted to help prevent flea infestation. Many owners also use ectoparasite control for their pets therefore it would have been challenging to enrol a large number of cats into this study who had not received any form of ectoparasite control. Future studies are required investigating UK stray cats in order to remove ectoparasite control as a potential cause for the low prevalence of *O. cynotis* reported in this study. This information

however may be less valuable to veterinary surgeons practicing in the UK who generally treat pet cats receiving regular prophylactic ectoparasite treatments. ~~An alternative method of detecting *O. cynotis* infection is the use of PCR[22] which could be evaluated in future studies. This may however be cost prohibitive in clinical practice and therefore trial treatment may be elected in the first instance. The life style cycle stage of the *O. cynotis* mite seen upon microscopy was not noted in this study.~~

Two out of the three cats were found to have live *O. cynotis* mites despite having received one application of ectoparasite control (Stronghold®: Selamectin and Broadline®: eprinomectin, fipronil, S-methoprene and praziquantel). One of these cats was a seven-week-old kitten who had received Stronghold® within four weeks of enrolment in the study, therefore clinicians should not discount *O. cynotis* based on previous acaricidal treatment alone. Unfortunately, the exact date of Broadline® application for the other cat was not recorded therefore the acaricidal application may been several weeks to months prior to sampling. One single application of eprinomectin, fipronil, S-methoprene and praziquantel has been shown to be effective in treating otoacariasis where one treatment corresponded to 96% preventive efficacy at day 28 based on ear mite counts.[23] A single application of selamectin was found to be 100% effective in resolving infestation 30 days after the treatment application in another study.[24] ~~Unfortunately, the date of ectoparasite administration was not recorded in this study.~~

Previous studies have found very different values for aural *Malassezia* counts in normal cats.[5,6,12] Two studies used the x 40 objective for examining each high power field.[5,6] In our study, similar to a previous study,[12] we used the x 100 oil immersion objective. Cytological methods have several limitations when compared to fungal culture. It is a method that is readily available to clinicians and gives semi-quantitative, immediate results. Limitations include inaccuracies in both cellular and microbial counts, operator dependency and reproducibility. Sometimes stain artefact was seen on slides which could easily be misinterpreted as infection if microorganisms were incorrectly noted (see figure S2 in Supplementary material). Some *Malassezia* organisms did not take up the stain so well therefore appearing as very ~~feint~~ faint structures which could easily be missed (see figure 2 Supplementary material**).** Seven species of *Malassezia* have been identified in the cat and of these most are lipid dependent therefore if fungal culture alone is used to detect *Malassezia* species in feline cerumen, lipid-dependent *Malassezia* species may go undetected as many laboratories only use mycological culture media without lipids.[9] In this instance, cytology may be more sensitive in detecting yeast infection.

Despite these limitations, ~~Within this cohort of cats,~~ those cats with otitis had five times as

many *Malassezia* per OIF than those with normal ears. The mean number for the otitis group was 0.687 (CI = 0.153 to 1.380) which equates to approximately one *Malassezia* per two OIFs. The mean number of *Malassezia* per OIF was 0.169 (CI = 0.114 to 0.228) in the group of cats without otitis, which equates to one *Malassezia* per six OIFs. It is important to note that some cats without clinical signs or otoscopic evidence of otitis externa had in excess of 10 *Malassezia* per OIF. ~~It is important to note that large numbers of *Malassezia* (>0.169 *Malassezia* per OIF) were found in some of the cats with normal external ear canals.~~ Therefore, if *Malassezia* are noted, this should be interpreted along with otoscopy findings and clinical signs of otitis. ~~The finding in this study of the~~ The presence of aural *Malassezia* in healthy cats in this study corroborated previous studies.[4-7]

One cat with *O. cynotis* and another cat with *D. gatoi* isolated, were found to have >10 and 7.8 *Malassezia* per OIF respectively, which is not surprising given that it may be an opportunistic microorganism as well as being part of the normal microflora. Interestingly, the ear with *D. gatoi cat* infestation had previously undergone a pinnectomy of the same ear. One ear with *O. cynotis* detected however did not have any *Malassezia* found upon cytology.

One cat from the otitis group referred to the Langford Small Animal Hospital with various comorbidities along with generalised exfoliative disease (*Malassezia* exfoliative dermatitis), was found to have very high numbers of aural *Malassezia* bilaterally (>10 per OIF), see figure S3 in Supplementary material. Unfortunately, this cat presented to the cardiology service at the Small Animal Hospital for congestive heart failure and further investigation including dermatohistopathology was not taken therefore the underling aetiology for the severe exfoliative dermatological disease was unknown. Other than echocardiography, further thoracic imaging was not performed therefore a thymoma could not be excluded. Previous studies have documented increased *Malassezia* in cats with concurrent illness.[12,25]

Two cats with large numbers (4+) of bacteria on cytology ~~(4+)~~ were associated with underlying aural pathology ~~such as otitis media and an aural mass~~ (bilateral otitis media and polyps in one cat and a unilateral aural mass in the other cat) documented using computed tomography (CT). One other cat with large numbers (4+) of bacteria unilaterally ~~(4+)~~ was found to have primary otitis externa and the underlying cause was not found. Only 6 / 341 cats ~~Small numbers of cats (n=6)~~ were found to have low numbers of bacteria (1+ or 2+) which is very different from previous studies where higher numbers of cats were found to have bacteria within the external ear canal.[5,6,10,11] These six cats with low bacterial counts were part of the non-otitis group (6 / 302). As bacteria were only noted cytologically in nine cats and two of these had a space occupying lesion present, mean bacterial values were not calculated.

~~It is important to note that large numbers of *Malassezia* (>0.169 *Malassezia* per OIF) were found in some of the cats with normal external ear canals. Although a mean was calculated for this grup, there was a range from 0 to >10 per oIF. Therefore, if *Malassezia* are noted, this should be interpreted along with otoscopy findings and clinical signs of otitis. It would also be prudent to take a cerumen smear to check for the presence of ectoparasites even if an acaricidal ectoparasite product is used.~~

A link between acne and *O. cynotis* has been reported.[20] The three cats identified as having *Otodectes* in this study did not have acne like lesions documented.

Only low numbers of saprophytes were found compared to a previous study,[10] most likely because most of the rescue cats were mainly housed indoors at time of sampling. The cats in this study were sampled throughout the spring and summer time. All nine of the cats where saprophytes were detected upon cytology had an indoor / outdoor lifestyle.

## Conclusions

Only a small number of cats were found to have *O. cynotis* in this study. If cats present for otitis, it is important to rule out ectoparasitic disease and to consider other causes of otitis in cats including allergic skin disease (non-flea non-food-induced feline hypersensitivity dermatitis, cutaneous adverse food reaction), space occupying aural lesions such as a polyp, neoplasia and otitis media (especially in cases of bacterial otitis). New mean values of *Malassezia* counts in the external ear canals of cats were documented in this study which may be a useful benchmark for those clinicians routinely performing ear cytology in cats.

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## Funding and Conflict of interest statements

Zoetis UK supplied some complementary anti-parasite products. S Tyler and N Barnard have received honorariums and consulted for Zoetis.

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## Supplementary material

| Breed | Number examined |
|---|---|
| Bengal | 1 |
| Birman cross breed | 1 |
| British Short Hair | 2 |
| Burmese | 1 |
| Devon Rex | 1 |
| Domestic Long hair | 23 |
| Domestic medium hair | 15 |
| Domestic short hair | 285 |
| Maine coon | 2 |
| Ragdoll | 1 |
| Russian Blue | 1 |
| Siamese | 3 |
| Siamese cross | 1 |
| Snowshoe | 1 |
| Somali cross | 1 |

| | |
|---|---|
| Missing data | 2 |

Table S1 Breeds of cats examined

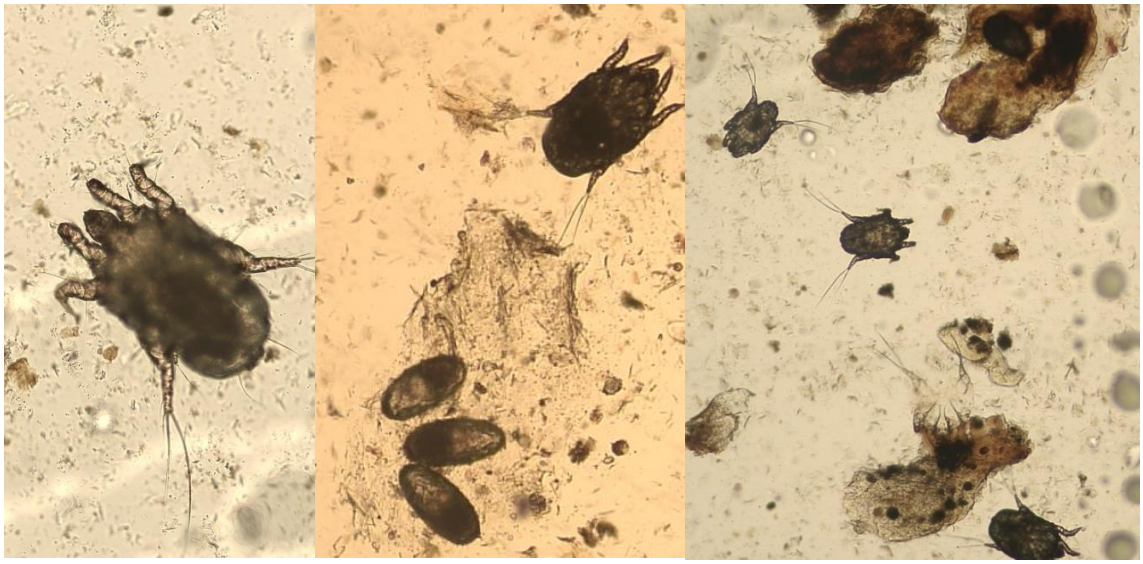


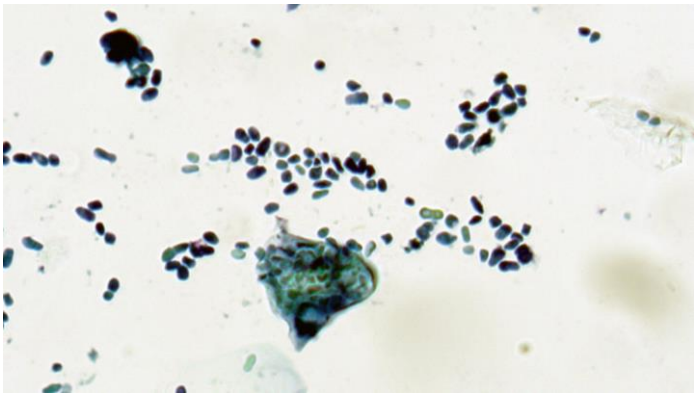
Figure S1 Microscopic evidence of *Otodectes cynotis* infestation
a) adult mite (100 x) b) one adult mite and three eggs (40 x) c) three nymphs (x 40)

| Case | Signalment | *Otodectes cynotis* visible upon otoscopy | *Otodectes cynotis* visible upon microscopy | *Demodex gatoi* visible upon microscopy | Ectoparasite control | Lifestyle | Exudate (quantity) | *Malassezia* (average number per OIF) |
|---|---|---|---|---|---|---|---|---|
| 1 | 3 years<br>FN<br>DSH | R YES<br>L YES | R YES<br>L YES | - | Broadline®*<br>(Eprinomectin<br>Fipronil<br>S-methoprene<br>Praziquantel)<br>once | Indoor / outdoor | R 3+<br>L 2+ | R 0<br>L 0.3 |
| 2 | 4 weeks<br>ME<br>DSH | R NO<br>L YES | R NO<br>L YES | - | - | Indoor | R 3+<br>L 3+ | R 2.6<br>L 1.6 |
| 3 | 7 weeks<br>ME<br>DSH | R YES<br>L NO | R YES<br>L YES | - | Stronghold®*<br>(Selamectin)<br>once within 4 weeks prior to sampling | Indoor / outdoor | R 3+<br>L 3+ | R > 10<br>L 7.3 |
| 4 | 12 years<br>FN<br>DSH | R NO<br>L NO | R NO<br>L NO | R YES<br>L NO | Stronghold®*<br>(Selamectin)<br>once | Indoor / outdoor | R 3+<br>L 3+ | R 7.8<br>L 0.2 |

Table S2 Otoscopy and aural cytology findings in cats with ear mites (*Otodectes cynotis* or *Demodex gatoi*)

503 R = right ear, L = left ear, OIF = oil immersion field, DSH = Domestic short hair, ME = Male
504 entire, FN = Female neutered, * = exact date of application prior to sampling unknown
505

506 a)



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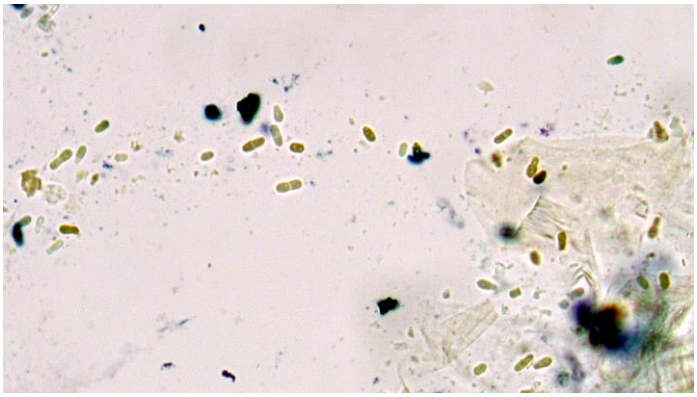
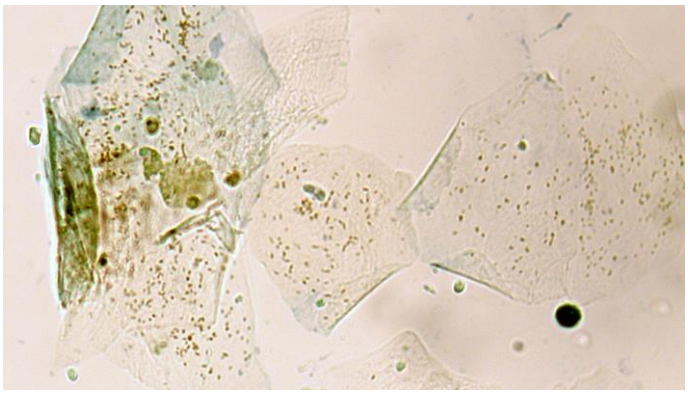


508 c)



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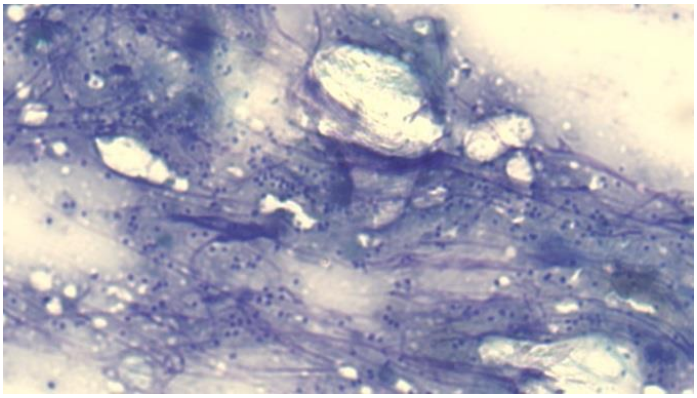
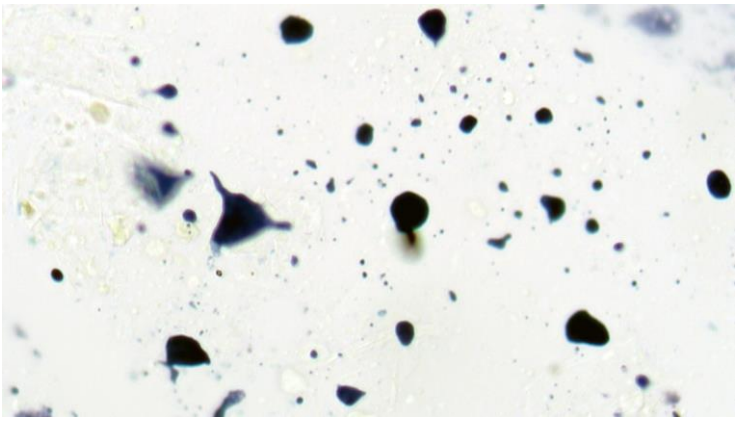


e)



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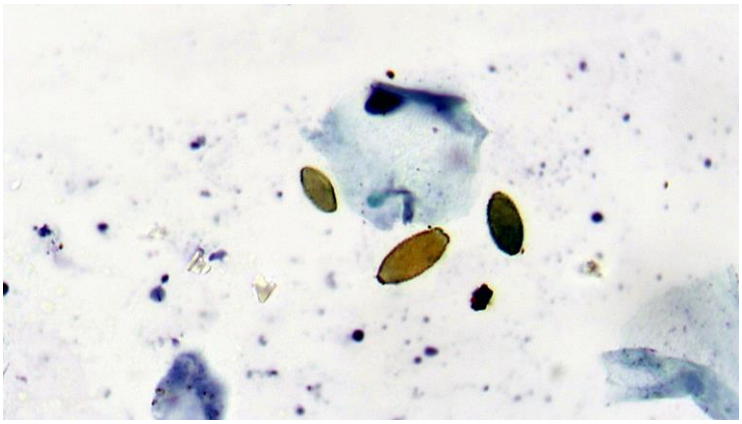

g)

Figure S2 Aural cytological findings

**a)** Stained *Malassezia*

**b)** Non-stained *Malassezia*

**c)** Keratinocytes containing numerous melanin granules

**d)** Degenerate neutrophils and nuclear streaming, with large numbers of coccoid shaped bacteria in a cat with purulent otitis externa in a protein rich background

**e)** Stain artefact

**f)** and **g)** Environmental likely fungal contaminants

| Case | Centre | Signalment | Reason for examination | Lifestyle | Otoscopy (erythema, ulceration, oedema) | Exudate (quantity) | Nature of exudate | Rod shaped bacteria (classification) | Coccoid shaped bacteria (classification) | Inflammatory cells | Clinical outcome |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Langford SAH | 16 years<br>FN<br>DLH | Study (Hyperthyroid assessment) | Indoor / outdoor | Unremarkable | R 2+<br>L 3+ | R dark brown<br>L dark brown | R 0<br>L 0 | R 0<br>L 1+ | R NO<br>L NO | No treatment recommended |
| 2 | Kats and Kits | 17 years<br>MN<br>DSH | Study | Indoor / outdoor | Unremarkable | R 3+<br>L 3+ | R brown<br>L cream, purulent | R 0<br>L 0 | R 0<br>L 2+ | R NO<br>L NO | Canaural® recommended |
| 3 | Kats and Kits | 10 years<br>MN<br>DSH | Study | Indoor / outdoor | Unremarkable | R 1+<br>L 1+ | R cream<br>L cream | R 0<br>L 0 | R 0<br>L 2+ | R NO<br>L NO | No treatment recommended |
| 4 | Bridgend CP | 1 years<br>MN<br>DSH | Study | Indoor / outdoor | Unremarkable | R 1+<br>L 1+ | R beige<br>L beige | R 0<br>L 0 | R 0<br>L 2+ | R NO<br>L NO | No treatment recommended |
| 5 | Bridgend CP | Unknown<br>FN<br>DSH | Study | Indoor / outdoor | Unremarkable | R 1+<br>L 1+ | R beige<br>L beige | R 0<br>L 2+ | R 0<br>L 2+ | R NO<br>L NO | No treatment recommended |
| 6 | Mayhew | 2 years<br>FN<br>DSH | Study | Indoor / outdoor | Unremarkable | R 2+<br>L 2+ | R cream<br>L cream | R 0<br>L 0 | R<br>L 1+ | R NO<br>L NO | No treatment recommended |
| 7 | Langford SAH | 4 years<br>FN<br>Siamese | Otitis externa (presented to the dermatology service) | Indoor / outdoor | Exudate obscured vision | R 1+<br>L 3+ | R beige<br>L haemorrhagic, purulent | R 0<br>L 0 | R 0<br>L 4+ | R NO<br>L YES | Resolution with topical and treatment and systemic glucocorticoids |
| 8 | Langford SAH | 10 months<br>MN<br>Maine coon | Otitis externa (presented to the dermatology service) | Indoor / outdoor | R 1+ erythema<br>L 2+ stenosis, polyp visible post flush | R 0<br>L 3+ | R none<br>L cream | R 0<br>L 4+ | R 0<br>L 4+ | R NO<br>L YES | CT scan and ear flush performed, surgery recommended. CT scan revealed bilateral otitis media and bilateral aural polyps within the middle ear |
| 9 | Langford SAH | 14 years<br>FN<br>DSH | Otitis externa (presented to the dermatology service) | Indoor / outdoor | R 2+ erythema and 2+ ulceration | R 2+<br>L 1+ | R brown<br>L yellow | R 0<br>L 0 | R 4+<br>L 0 | R NO<br>L NO | CT revealed mass at junction of vertical and horizontal ear canal, surgery recommended |

## Table S2 The otoscopic and cytological findings of cats with bacteria found on aural cytology.

R = right ear, L = left ear, DSH = Domestic short hair, ME = Male entire, FN = Female neutered, MN = Male neutered

Figure S3 Cat with generalised exfoliative disease (aetiology unknown) large numbers of *Malassezia* noted upon cytology (>10 per oil immersion field)