Cytology of the external auditory meatus in healthy domestic pet rabbits (Oryctolagus cuniculus)

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SUMMARY

The cytological examination of healthy external auditory meatus from 146 pet rabbits (108 with erect pinnae and 38 with pendulous pinnae) showed the presence of Malassezia yeasts in 54% of the animals. Yeasts were detected in both meatus in 72 cases and only one meatus in 26 cases. There was no correlation with age, sex or ear anatomy (straight or lop ears). In the animals showing the presence of Malassezia yeasts, the number of yeasts per high magnification field (obj. X100) varied from 1 to 60 (mean: 11). We can conclude from this study that the presence of Malassezia yeasts in rabbit external auditory meatus is physiological and that the frequency of these yeasts in non inflammatory ear meatus of domestic rabbits is comparable to what is observed in other mammalian species.

Keywords: Rabbit, ear, cytology, Malassezia

Material and Methods

STUDY POPULATION

All pet rabbits presented to the Exotic Animals Service of the Advetia Veterinary Clinic (Paris, France) in 2013, were considered for the study regardless of the presenting reason for consultation. The criterion for inclusion was the absence of history and clinical signs of otitis externa, confirmed by video-otoscopic examination.

SAMPLE COLLECTION

Samples were obtained using a narrow non-sterile cotton-tipped applicator (Ecouvillon sec Copan tige aluminium). The rabbit was held by an assistant, the investigator inserted a swab into the ear meatus carefully leading it to the proximal area. The swab was rotated three times 360º and removed.

SAMPLE PROCESSING

Each of the cotton swabs from both ears of each rabbit was then slowly rolled once on to a glass slide. The glass slides were air-dried for 10 minutes, fixed and stained with a modified Wright's stain kit (RAL555, RAL Diagnostics France) as prescribed by manufacturer. Using this staining technique, bacteria and fungal elements are stained in blue [7].
SPECIMEN EVALUATION

All the samples were analyzed by the same investigator (MF) using a translucent upright microscope (Eclipse E200; Nikon Microscopes, France). Examination started at low-power (x40) magnification (obj. x4) to identify a representative and easily evaluable area. Microorganisms were counted on eight fields with immersion oil high power magnification (x100).

STATISTICAL ANALYSIS

Data were analyzed by using the chi-square test to determine breed and age-related variations and the Wilcoxon Mann-Whitney test to compare the number of yeasts between lop and straight ears. A P value of < 0.05 was considered significant.

Results

STUDY POPULATION

We examined 146 dwarf rabbits without otitis externa: 78 males and 68 females, age ranging from 3 months to 11 years (mean 4.5 year). Thirty eight of the rabbits had pendulous pinnae (lop eared rabbit) and 108 had erect pinnae.

SAMPLE EVALUATION

The results of the microscopic evaluations are summarized in Table I. The cytological evaluations of the samples revealed the presence of unipolar building yeasts (Fig 1). These morphological characteristics were in accordance with Malassezia yeasts, most probably M. cuniculi [4]. Yeasts were spherical with rare small spherical buds and large size variations (2-5 µm).

Malassezia yeasts were detected in 170 meatus (54%): both meatus in 72 cases and only one meatus in 26 cases (25%). In the animals showing the presence of Malassazia yeasts, the number of yeasts per high magnification field (obj. X100) varied from 1 to 60 (mean: 11). Less than 5% of rabbits showed more than 30 yeasts per field.

There was no significant difference regarding yeast isolation or number between the ear pinnae types (Fig.2), sex and age groups (Fig. 3):

<table>
<thead>
<tr>
<th>Yeasts</th>
<th>Bacteria (cocci)</th>
<th>Inflammatory cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erect pinnae (straight ears) (n=108)</td>
<td>56% (60/108)</td>
<td>6% (6/108)</td>
</tr>
<tr>
<td>Pendulous pinnae (lop ears) (n=38)</td>
<td>47% (18/38)</td>
<td>10% (4/38)</td>
</tr>
</tbody>
</table>

Table I: Percentage of presence of yeast, bacteria and inflammatory cells counted on 8 high magnification fields (obj.x100)
No rods were detected in any sample but cocci were detected in 10 cases (6 unilateral, 4 in both ears, <10 bacteria per high magnification field) (Table 1).

Discussion

The presence of Malassezia yeasts has rarely been reported in rabbits. The results of this study are in agreement with two previous studies identifying Malassezia sp on the skin, hair, and ear meatus of rabbits. Cabanes et al. [3] isolated M. cuniculi from one ear out of 11 laboratory rabbits. In a study of occurrence of Malassezia species in different mammals and birds, Dufait [10] described the isolation of M. pachydermatis from rabbits. In the present study, the shape of the yeast isolated on rabbit ear cytology was compatible with M. cuniculi as previously described in samples from New Zealand white rabbits: spherical with rare small spherical buds and large size variations (2-5 µm) [4]. Other species of spherical Malassezia, such as M. globosa are described in other species (man, cow, cheetah) [3]. Malassezia caprae, M. dermatis, M. furfur, M. japonica and M. restricta can also be spherical but are mainly observed as a mixture of ellipsoidal and spherical yeasts [3, 13]. A definitive species identification would need culture and DNA sequence analysis [15] which were not included in the present study.

The frequency of detection of yeasts from the ear swabs was higher than expected according to previous studies considering Malassezia yeasts as rare in lagomorphs [4, 14]. As yeasts can be observed in almost half of the samples, we can consider Malassezia colonization of ear meatus as physiological in domestic pet rabbits. Cytology being less sensitive than fungal culture, and counting done on only 8 fields, colonization is probably more frequent than 50%. Discrepancies with previous reports can be due to the nature of population studied (pet dwarf rabbits in our study and New Zealand rabbits which were used for breeding and meat production in other studies) and environment (human homes in our study versus laboratory environment in other studies).

The frequency of Malassezia yeasts in non-inflammatory ear meatus of domestic rabbits is comparable to what is observed in other mammalian species like dogs [5, 8, 17, 19, 21], cattle, horses, sheep and goats [9]. As in other animal species Malassezia yeasts seem to be a commensal fungal element of the external ear. It is also a normal element of skin flora and can proliferate in some skin inflammatory diseases in rabbits (i.e. sarcoptic mange) [18].

Incidence of Malassezia otitis in pet rabbits is unknown. Further studies are needed to appreciate the prevalence of Malassezia infection in external otitis and otitis media in pet rabbits and its potential pathological role.

Bacteria were rarely observed and always in very small number. This is quite different compared to canine and feline ear cytology where cocci are common [19, 20, 21].

As in the dog there was no difference between erected and pendulous pinnae in this study [7]. This is comparable to what is observed in dogs ear pinna shape does not influence ear meatus temperature [16]. Only the amount of hair influenced temperature and Malassezia concentration in healthy canine ears [12, 16]. All the rabbits were living in apartments in temperate climate (Paris and its suburb, France). Incidence of bacterial or fungal colonization in more humid and warmer climates could probably be higher as shown in dogs and cows [8, 9].

Conclusion

This study should encourage practitioners to systematically use cytology in case of external otitis in the rabbit. This would help to chose the proper topical treatment and limit the risk of development of chronic external otitis or otitis media.

References