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Detection of *Malassezia* spp. from healthy cattle and cattle with otitis by direct examination and culture

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ABSTRACT

The occurrence of bovine external otitis in tropical regions is predominantly assigned to parasitic infestations. These infections represent a significant problem and can be associated with vestibular disease and death. Yeasts of the genus *Malassezia* are members of the normal microbiota on human and animal skin, and may also be associated with bovine parasitic otitis. The purpose of this study was to evaluate the prevalence of the genus *Malassezia* in healthy cattle and cattle with otitis by direct microscopic examination and culture. Specimens of 1010 cattle were collected with sterile swabs, inoculated onto Mycosel medium, supplemented with olive oil, and incubated at 32ºC for one week. In addition, 200 cattle (143 healthy and 57 with otitis) were also evaluated by direct microscopic examination using a Gram staining method to detect *Malassezia* spp.. Using the direct examination of the specimens no significant difference was observed between the presence of *Malassezia* spp. from 143 healthy animals (30.7%) and from 57 animals with otitis (26.3%). Culture was positive for *Malassezia* spp. in 329 (38.12%) of a total of 863 healthy cattle and in 93 (63.27%) of 147 cattle with otitis ($p<0.001$). In this study the detection of *Malassezia* spp. from cattle with a high level of confidence was possible by culture. This exam was much more specific, allowing a much more accurate visualisation of yeast cells and giving isolates for biochemical tests.

Key words: *Malassezia* spp., otitis in cattle, diagnosis.

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INTRODUCTION

In tropical regions, otitis in cattle has been predominantly attributed to parasitic infections caused by rhabditiform nematodes. These infections represent a significant problem, especially in Gyr cattle, which may eventually die as a result [4,13,14]. Bovine otitis may also be caused by acarids of the genus *Raillietia* which are found at a high frequency in Nellore and Guzera cattle, and in European breeds [1,7].

In a previous study the identification of *Malassezia* spp. by direct microscopy was possible in 24 of the 50 specimens obtained from the ears of healthy cattle. In primary cultures was recovered *Malassezia* spp. from eight samples which were classified at that time as *P. ovale* [11]. One study of yeast in cultures using Sabouraud's medium from the skin of six cows revealed positivity in two cases, and the isolates have corresponded to the lipid-independent species, *M. pachydermatis* [6]. Using direct microscopy, one study of specimens from the skin and ears of 55 healthy cattle has revealed the presence of *Malassezia* spp. in 16 clinical specimens [9].

The genus *Malassezia* were isolated in 34.6% of the cultures of specimens from the external auditory canal of healthy cattle (378), and in 54.7% from the animals with external otitis (n=75). Lipid-dependent species were the most frequent [3]. In purely mycological studies the genus *Malassezia* was the fungus most frequently isolated from cattle with parasitic otitis [5].

The purpose of this study was to evaluate the prevalence of the genus *Malassezia* in healthy cattle and cattle with otitis by direct microscopic examination, using the Gram staining method, and by culture.

MATERIALS AND METHODS

The sample for this study consisted of 1010 cattle, of which 863 were healthy and 147 diagnosed with otitis, from farms located in the State of Minas Gerais, Brazil.

Cerumen or secretion from one ear of each animal (either left or right) was collected from the external ear close to the external acoustic meatus. These specimens were collected with the aid of sterile swabs and immediately inoculated into a test tube containing Mycosel agar medium[1], modified by the addition of glucose[1] (final concentration 4%) and chloramphenicol[2] (final concentration 150 mg/l), with olive oil added to the surface of the medium. Incubation was carried out at 32°C for seven days [3,5]. The isolation of yeasts of the genus *Malassezia* was confirmed by direct observation of the colonies, and microscopic identification of cells by the Gram staining method with their characteristic broad base unipolar budding [12].

The swabs obtained, still containing clinical material left after seeding, were then placed in test tubes and exposed to sunlight for parasitological diagnosis [4,13].

In addition to the determination of the prevalence of *Malassezia* spp. by means of culture, 200 cattle (143 healthy and 57 with otitis) were also evaluated by direct microscopic examination using Gram staining method to detect this yeast.

The Chi-square test was carried out to determine the existence or not of statistically significant differences in the sampled groups. Differences with $p < 0.05$ were considered significant.

RESULTS

In the parasitological tests, among animals with otitis (n=147), the presence of rhabditiform nematodes was detected in 127 Gyr cattle and acarids of the genus *Raillietia* were detected in 20 cattle (eight Holstein and 12 hybrid). Culture was positive for *Malassezia* spp. in 329 (38.12%) of a total of 863 healthy cattle and in 93 (63.27%) of 147 cattle with otitis ($p<0,001$).

Direct examination of the ear secretions from 57 animals with otitis permitted the observation of predominantly Gram positive cocci in 21 slides, bacillus cocci in eight and Gram positive rods in 25. In three samples it was not possible to describe the predominant forms of bacteria due to a large quantity of cellular debris and coloured artefacts produced by the remains of parasites which interfered with the microscopic image. Yeasts with micromorphology corresponding to *Malassezia* spp. were seen in 15 (26.3%) slide samples collected from animals with otitis. Of these 15 positive specimens by direct examination, eight were confirmed by culture growth. For 23 samples from animals with otitis which showed positive culture, the direct examination of these specimens did not allow the visualisation/ detection of the genus *Malassezia* (Table 1).

Yeasts with characteristics similar to the genus *Malassezia* were seen in 50 (35%) slides of specimens collected from 143 asymptomatic animals. Positive culture was possible for yeast in 44 (31%) of the specimens from these animals. The statistical analysis of the data showed no differences between the numbers of positive exams for *Malassezia* spp. coming from cattle with and without otitis when only direct examination was used. However culture revealed a significant difference where $p<0.001$ between the groups: 54.4% positive in cattle with otitis and 31.8% in healthy cattle (Table 1).

Table 1. The occurrence of yeasts of the genus *Malassezia* observed by direct examination using Gram staining method and by culture of specimens collected from the external ear canal of 143 healthy cattle and 57 with otitis.

Results	Healthy n(%)	With otitis n(%)
Positive cultures	44 (31.8)	31 (54.4)
Negative cultures	48 (33.6)	17 (29.8)
Contaminated cultures	51 (35.6)	9 (15.8)
Posit. direct exams and cultures	26 (18.2)	8 (14.1)
Positive direct exams	50 (35)	15 (26.3)
Negative direct exams	93 (65)	42 (73.7)

DISCUSSION

The examination of clinical otitis in 1010 cattle from the State of Minas Gerais, Brazil has shown positivity for rhabditiform nematodes in 127 Gyr cattle and acarids of the genus *Raillietia* were detected in twenty cattle. These results reveal that parasitic otitis is still present in the cattle of Brazil, particularly in the Gyr breed.

Culture was positive for *Malassezia* spp. in 329 (38.12%) of a total of 863 healthy cattle and in 93 (63.27%) of 147 cattle with otitis. These data demonstrate a higher prevalence of *Malassezia* spp. among the cattle with otitis. The higher prevalence of *Malassezia* spp. in Gyr cows with otitis may indicate the contribution of these microorganisms in the aetiology of parasitic otitis caused by rhabditiform nematodes. These yeasts could be producing virulence factors such as proteases, condroitin-sulphatase, phospholipases and hialuronidases, which have already been described for *M. pachydermatis* [2], thus producing or increasing the inflammatory reaction in the external ear. As a hypothesis, the yeasts of the genus *Malassezia* may be degrading substrates, principally the lipids present in cerumen, facilitating the nutrition of nematodes as well as bacteria present at the site of infection. They may be stimulating the over production of cerumen and therefore increasing the amount of overall nutrients. Virulence factors and the presence of antigens to yeast may be the stimulus for increased secretions.

Direct microscopic examination by Gram staining of the specimens collected from animals with otitis (n=57) revealed a great diversity of bacterial groups present in ear secretions. Rods and Gram-positive cocci made up the bacterial forms most common in the smears of secretions from these animals.

Using direct examination of the specimens, no significant difference was observed between the presence of *Malassezia* spp. from 143 healthy animals (30.7%) and from 57 animals with otitis (26.3%). However, through the culture of specimens from the same 57 cattle with otitis we observed 54.4% positive results for *Malassezia* spp., demonstrating that this method is more sensitive.

The presence of yeasts of the genus *Malassezia* observed by direct examination in 15 animals with otitis was confirmed by cultivation of eight clinical specimens. The absence of these microorganims in the cultures from the remaining seven samples (12.3%) are considered false negatives. This may be explained by the presence of non-vital yeast cells or perhaps they were inhibited or incapable of growth in the cultivation medium by the presence of contamination. Of the 23 specimens collected from animals with otitis which resulted in positive culture, direct examination of specimens collected did not permit the observation of yeasts of the genus *Malassezia*, which represented 40% of the false negative results using direct examination. Coloured artefacts, the presence of bacteria and debris from the inflammatory process complicates the direct observation of secretions from the ear, which contributes to both false positive and negative results. A further problem for direct examination is that aspects of colour that are important for the microscopic classification of yeasts of the genus *Malassezia* are often incomplete, since different species can present similar forms [8,10].

Culture is much more specific, allowing a much more accurate visualisation of yeast cells and giving

isolates for biochemical tests which may be used for confirmation and identification of yeasts of this genus. Once reconstituted the Mycobiotic agar liberates cycloheximide in a concentration of 0.05 which efficiently inhibits the growth of mycelial fungi. In addition the film of olive oil helps to counter the growth of other contaminants (rhabditiform nematodes, other yeasts and mycelial fungi such as *Trichophyton* spp.) which are tolerant to cycloheximide.

The *Malassezia* spp. prevalence in healthy cattle by direct examinations was quite close to that reported by Guillot *et al.* [9] in material collected from skin and the external auditory canal (29%). Gustafson [11] described a higher prevalence (48%) for specimens collected from the external auditory canal of healthy cattle. Yeasts of the genus *Malassezia* have been detected in significant frequencies in healthy cattle and may be members of the ear's resident mycobiota [3].

The detection of yeasts of the genus *Malassezia* from ears of cattle by culture in this study showed a high confidence level. Using this method it was possible to confirm a significantly higher occurrence of this yeast in specimens from the ears of cattle with otitis. This result shows the superiority of culture in the detection of *Malassezia* spp. from the ears of cattle or other domestic animals with otitis compared to other available technique.

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Sources and Manufacturers

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