

Cytological Features of the Normal Ear Canal of Wild Jackals (*Canis aureus*) and Domesticated Dogs (*C. domesticus*)*

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Received February 16, 2012; revised March 17, 2012; accepted March 26, 2012

ABSTRACT

This is the first reported study in which various cytological and microbial components of the ear canal of wild jackals (*Canis aureus*) were examined and compared with those of domesticated dogs (*C. domesticus*). It is proposed that the differences between them might be attributable to domestication. The normal cytology of the jackals' ears includes cerumen, keratinous debris, coccoid bacteria and yeast-like organisms similar to domesticated dogs, but the frequencies of these findings differed significantly between the two species. In the jackals the incidences of ceruminous debris and yeasts were significantly lower ($p < 0.001$, $p = 0.004$ respectively), while keratinous debris and coccoid bacteria were significantly higher ($p < 0.001$). During domestication some changes have probably occurred in the dogs' lifestyle that predisposed them to the growth of yeasts in their ears but less to bacterial growth. It is possible that the higher numbers of bacteria might be a result of environmental contamination, because some of the jackals lived near urban centers and feed on garbage.

Keywords: Canine; Golden Jackal; Otitis Externa; Cocci; Yeast

1. Introduction

The prevalence of ear infection in wild canids is unknown, nor is the presence and number of infectious agents in their ears. In humans the prevalence of otitis media is very high compared with wild species [1]. In domesticated dogs otitis externa is very common and is estimated to occur in 10% - 20% of dogs. The most common microorganisms isolated from the ears include the bacteria, *Staphylococcus intermedius* (now reclassified as *Staphylococcus pseudintermedius*), *Pseudomonas aeruginosa*, and—*Proteus* species, and yeasts such as *Malassezia pachydermatis* [2-5]. Most of the microorganisms isolated from inflamed ears are found in smaller numbers in normal ears [5-9]. In the dog, primary causes and predisposing factors, such as allergies, keratinization disorders and anatomical abnormalities, create suitable conditions for the proliferation of microorganisms [9,10]. The role of domestication and human intervention in creating

some of these problems is evident (*i.e.* breed-associated abnormalities), while other primary causes such as allergies or keratinization disorders can also be attributed to domestication. However it is questionable whether the spectrum of microorganisms in the normal canine flora is also due to domestication, and it is also unclear whether wild and domesticated canids share similar microorganisms. In order to approach this problem, we have compared cytological parameters in the ear canals of wild canids as represented by the golden jackal (*Canis aureus*) with the domesticated dog. The jackal, which is the commonest free-ranging wild canid in the region, was chosen because of the relative ease of sampling its ear canals.

2. Materials and Methods

2.1. Sample Collection

Fresh cadavers of 81 golden jackals presented at the pathology department of the State Veterinary Institute were examined during the years 2007-2009. Samples from both ears, 162 in total, were taken with cotton swabs inserted into the depth of the ear canals and evaluated cytologically.

*Conflict of interest statement: The authors declare that there is no conflict of interest.

Ethical standards: Ethical approval was not needed for this study, as the authors have sampled cadavers of animals that were brought to the pathology department following natural death or animals that were culled by governmental authorities as part of a regional public health program for rabies control.

Smears were also taken from each ear of 49 fresh dog cadavers brought to the same institute for various reasons including stray dogs during part of the same period. A total of 98 ear canal samples were taken similarly to the jackals. Dogs from breeds with known anatomical features that predispose them to otitis externa were excluded from the study [5,9,11,12]. Most of the dogs were mix breeds and stray dogs. Dogs with a history of antibiotic or glucocorticoids treatment prior to their death were excluded. In both species only cadavers without skin problems or apparent ear problems as exhibited by erythema of the pinnae and the canal orifices, or excessive secretions from the canals were sampled.

Ethical approval was not needed for this study, as the authors have sampled cadavers of animals that were brought to the pathology department following natural death or animals that were culled by governmental authorities as part of a regional public health program for rabies control.

2.2. Cytological Examination of Ear Canals

The samples were heat-fixed and stained with Diff Quick® (Jorgensen Laboratories Inc., Loveland, CO, USA). All smears were examined by the first author and evaluated microscopically for the presence of cerumen and keratinous debris at a magnification of 100×. A magnification of 1000× (high power field-HPF) was used to examine for the presence of inflammatory cells, bacteria and yeasts. In areas with the highest concentrations of material, at least 10 HPF's were examined. All of the studied cytological parameters were graded on a scale of 0 to 4. For endogenous cell assessment and cerumen the score was subjective (0: not present; 1: very few; 2: low amount; 3: moderate to high amount; 4: very high amount), and for the infectious agents scoring was semi-quantitative on a scale from 0 to 4 (0: not present; 1: 1 to 3 organisms/HPF; 2: 4 to 10 organisms/HPF; 3: 11 to 30 organisms/HPF; 4: over 30 organisms/HPF). This scoring system was routinely conducted in the first author's practice.

2.3. Microbial Culture

From 3 jackals which yielded bacteria on cytological smears, samples were also taken for microbial culture.

Samples were inoculated directly onto sheep blood agar (Columbia agar base supplemented with 5% sheep blood), and a MacConkey plate for gram-negative bacteria. Identification of the bacteria was carried out based on colony and microscopic morphology, plasma coagulase test and biochemical characteristics by standard methods [13].

The samples were also inoculated on Sabouraud dextrose agar.

2.4. Statistical Analysis

The Chi-Square and Fisher's exact tests were applied for assessing the associations between two categorical variables. This included testing the associations between jackals and dogs for the various cytological parameters (*i.e.* ceruminous and keratinous debris, cocci, rods and *Malassezia*), and within each species the associations between ceruminous debris and *Malassezia* and between keratinous debris, cocci and rods.

In addition to these measurements performed on both ears of one jackal or one dog were correlated. Therefore, analysis of each variable was performed using a generalized estimating equation (GEE) regression model (SPSS Inc. 2007) with an exchangeable covariance structure for the working matrix (*i.e.* correlation between measurements of two ears in the same animal but not between animals). The Wald Chi-Square statistic was used to compute statistical significance and 95% confidence intervals. Marginal means were calculated from the model and were presented in the relevant tables.

All tests applied were two-tailed and a *p*-value of 5% or less was considered statistically significant.

Analyses were carried out using the SPSS statistical software (PASW statistics version 18, 2010).

3. Results

Results of all cytological parameters are presented in **Table 1**.

Ceruminous debris—the jackals had a significantly lower frequency than the dogs (113/162 ears (70%) vs 42/98 (43%), scored "0") and they also scored lower cytologically (3/162 scored "4" vs. 5/98; 7/162 scored "3" vs. 17/98) ($p < 0.001$).

Keratinous debris—most of the jackals did not have keratinous debris (83/162 ears vs. 31/98 ears scored "0"). However, they had a higher frequency at the highest levels of keratinous debris; in 8 jackals, a score of "4", while none of the dogs scored "4" ($p < 0.001$, Wald Chi Square $p = 0.019$).

Almost half of the jackals' ears (76/162 (47%)) had cocci while only 13/98 ears (13%) of the dogs had cocci. Furthermore—scoring of "3" and "4" were found in 6 (3.7%) and 3 (1.8%) jackals' ears respectively, and only one ear of a dog scored "3" and none "4" ($p < 0.001$).

The vast majority of dogs (94/98) did not have rods, which, in contrast, were present in 23/162 (14%) ears of jackals. Only 2 of the jackals' ears scored "4" for the presence of rods ($p = 0.044$, Wald Chi Square $p = 0.016$).

More dogs' than jackals' ears had *Malassezia* or yeast-like microorganisms in them (15 jackals—9% and 24 dogs—24.5%), and they also scored higher ($p = 0.004$).

Neutrophils were not found in any of the examined dogs' ears and were found in only 3 jackals' ears. This

Table 1. Scoring of cytological findings in ear canals of jackals and dogs. Number of ears and (percentage) within species.

Cytology Parameter and Levels ^a	Cerumen ^b		Keratinous Debris ^c		Cocci ^d		Rods ^e		<i>Malassezia</i> ^f		Neutrophils	
	Jackal	Dog	Jackal	Dog	Jackal	Dog	Jackal	Dog	Jackal	Dog	Jackal	Dog
0	113 (69.7)	42 (42.9)	83 (51.2)	31 (31.6)	86 (53.1)	85 (68.7)	139 (85.8)	94 (95.9)	147 (90.7)	74 (75.5)	159 (98.1)	98 (100)
1	24 (14.8)	17 (17.3)	42 (25.9)	37 (37.7)	54 (33.3)	6 (6.1)	13 (8.0)	1 (1.0)	12 (7.4)	17 (17.3)	1 (0.6)	0
2	15 (9.3)	17 (17.3)	17 (10.5)	25 (25.5)	13 (8.0)	6 (6.1)	5 (3.1)	1 (1.0)	2 (1.2)	6 (6.1)	1 (0.6)	0
3	7 (4.3)	17 (17.3)	12 (7.4)	5 (5.1)	6 (3.7)	1 (1.0)	3 (1.8)	2 (2.0)	1 (0.6)	1 (1.0)	0	0
4	3 (1.8)	5 (5.1)	8 (4.9)	0	3 (1.8)	0	2 (1.2)	0	0	0	1 (0.62)	0

^aScoring levels: refer to Materials and Methods section; ^bChi Square $p < 0.001$ Wald Chi Square $p < 0.001$; ^cChi Square $p < 0.001$ Wald Chi Square $p = 0.019$; ^dChi Square $p < 0.001$ Wald Chi Square $p < 0.001$; ^eChi Square $p = 0.044$ Wald Chi Square $p = 0.016$; ^fChi Square $p = 0.004$ Wald Chi Square $p = 0.004$.

finding was similar between groups ($p = 1.00$).

Culture results showed mixed bacterial growth in all the submitted samples. However specific bacterial isolation could not be performed. No yeasts were grown on Sabouraud dextrose agar.

There was a significantly high correlation between levels of keratinous debris and cocci in the jackals ($p = 0.004$), but not in the dogs ($p = 0.49$). There was a significantly high correlation between levels of ceruminous debris and *Malassezia* in the dogs ($p = 0.002$) but not in the jackals ($p = 0.42$).

4. Discussion

This is the first report of the cytological and microbial components in ear canals of wild canids compared with domesticated dogs. In almost all of the examined parameters the wild canids, represented by the golden jackal, were significantly different from the domesticated dogs. The jackals had significantly less ceruminous debris and yeasts' like organisms in their ear canals. It is expected that domestication and especially the pronounced phenotypical diversion of dogs would create favorable conditions for the proliferation of bacteria and yeasts. It has been reported that pendulous pinnae, hyperplastic ceruminous glands and congenitally stenotic ear canals might contribute to elevations in temperature and/or humidity, which create suitable conditions for the proliferation of yeast and bacteria in the ear canal [5,12]. These anatomical variations, however, are not present in jackals. In this report the dogs had similar presence of bacteria and yeasts in their normal ear canals as reported previously [5-8]. The most unexpected results were the significantly higher presence of bacteria in the jackals' ears. Unfortunately the bacteriology results in the present study showed a mixed bacterial growth and specific bacterial isolates could not be identified. Ideally, we should have submitted samples for bacterial culture from all the cases in

which bacteria were found cytologically. However this was not feasible in this study.

The relatively high levels of bacteria in jackals' ears could be due to environmental contamination. The jackals in this report came partly from areas in which they lived in close proximity with civilization and feed on garbage and external contaminants could have gained access to the depth of the ear canals. It is interesting that a few jackals also had neutrophils in their ears, which were not found in any of the dogs' ears. This finding, although not statistically significant, can contribute to the possibility that the lifestyle of the jackals enables contamination and inflammation, albeit not noticed in the gross appearance of the ears. Some of the dogs were strays that probably also lived and fed on garbage, and did not show environmental contamination. It can be speculated that domestication helped with this kind of protection. It is also possible that the higher production of cerumen in dogs as compared to jackals plays a role in this protection. Moreover, the high amount of cerumen was well correlated with high numbers of *Malassezia* in dogs.

The findings of this study indicate whether domestication has played a role in the reported normal flora of dogs' ears. The jackal in this study represented wild canids, and because of the relatively easy access of sampling ear canals of this species which is the commonest free-ranging wild canid in the region and is genetically related to the dog [14]. Jackals can serve as a model for this kind of comparative research due to their relative ubiquity in many places.

The jackals and the dogs in this study did not show any gross pathology of their skin or ears. It has been recently reported that most of examined normal jackals, coming from the same geographical area as those in the present study, were PCR-positive for *Leishmania tropica* which is endemic in the area [15]. Jackals also have other pathogens similar to dogs, like canine distemper, parvovirus and *Ehrlichia canis* [16,17].

5. Acknowledgements

The authors would like to thank Drs. Dan Lahav and Nir Edery from the Pathology department at the Kimron Veterinary Institute for their help in providing the study animals, and Prof. Danny Elad from the Microbiology department at the Kimron Veterinary Institute for the microbiology analysis.

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