



Report of Mollicutes in the Ear Canal of Domestic Dogs in Brazil

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Abstract

Several microorganisms as bacteria, fungi, yeast and ectoparasites compose canine ear canal microbiota. These agents can be native or invasive pathogens that cause clinical otitis. The aim of this study was to associate Mollicutes in external ear canal with clinical otitis. 41 domestic dogs were examined, and a total of 82 otologic samples were collected using sterile swabs, placed in a plastic storage tube containing 2 milliliters of sterile phosphate buffer saline (PBS, pH 7.2) and appropriately labeled. The samples were processed for isolation of Mollicutes using Hayflick's medium. The frequency of Mollicutes class in the isolation was 32.9% (27/82). 14.8% (4/27) of the dogs, which were positive for Mollicutes, had external otitis and 85.2% (23/27) were healthy dogs. The genus *Mycoplasma* spp. was detected in 7.4% (2/27) by digitonin test. It was the first isolation of Mollicutes from the external ear canal in dogs. Although there have been important findings in this study, the presence of these bacteria cannot indicate the onset and perpetuation of external otitis in dogs.

Keywords

Mollicutes, Otitis, Canines, Microbiota, Brazil

Subject Areas: Microbiology, Veterinary Medicine

1. Introduction

Microbiota of the ear canal in dogs is composed up of commensals and opportunists microorganisms. Bacteria of the genus *Staphylococcus* spp., *Streptococcus* spp. *Corynebacterium* spp., *Proteus* spp. are often found as a component of the normal flora of the ear canal, however unstable conditions may cause clinical otitis [1]. Identifi-

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fied *Staphylococcus intermedius*, *Staphylococcus aureus* subsp. *aureus*, *Pseudomonas* spp., *Proteus mirabilis* and *Klebsiella pneumoniae* in dogs with severe otitis [2]. Many authors have described *Staphylococcus* spp. as the most common bacteria in otitis cases [2]-[6]. However, up to 35% of the dog's clinical otitis isolation are *Pseudomonas* spp. and Gram-negative bacteria that not constitute the normal microbiota [7]-[9].

Mollicutes class is the smallest prokaryotes reported with replication capacity, and it is the most fastidious bacteria found in nature, that requires complex molecules such as cholesterol, sterol and urea for growth. These microorganisms are found in avian, human, insect, mammalian, plant and reptilian hosts. Some species are pathogenic and have been associated with different infections such as anemia, arthritis, otitis, infertility and respiratory disease [10]-[12]. Mycoplasmas in dogs has been described [13]. Nowadays, 15 species are known and two species with incompletely described. Species of mycoplasmas detected or isolated in dogs are: *Acholeplasma laidlawii*, *Mycoplasma arginini*, *Mycoplasma bovigenitalium*, *Mycoplasma canis*, *Mycoplasma cynos*, *Mycoplasma edwardii*, *Mycoplasma feliminutum*, *Mycoplasma felis*, *Mycoplasma gateae*, *Mycoplasma haemocanis*, *Mycoplasma maculosum*, *Mycoplasma molare*, *Mycoplasma opalescens*, *Mycoplasma* sp. HRC689, *Mycoplasma* sp. VJC358, *Mycoplasma spumans* and *Ureaplasma canigenitalium* [14] [15].

Some species detected in canine have been isolated in others hosts, though the canines' mycoplasma flora were not be fully described. This report refers to species that occur in the respiratory and reproductive tract, oral and nasal cavity, conjunctiva, pericardial sac, kidney, liver, lymph node, spleen, bladder, cerebral spinal fluid, blood, synovial membrane [16]-[19]. Several authors described canines' mycoplasmas species in different parts of the body [14] [19]. No previous studies of Mycoplasmas found the bacteria from the ear canal of dogs. The present study was conducted to investigate members of the Mollicutes class in the ear canal of canines with and without otitis.

2. Material and Methods

2.1. Animals and Samples

Domestic dogs from different localities attended veterinary hospital of the Federal Rural University-UFRPE, Recife-PE. 41 dogs at different ages and breeds, females and males were used in this study. Anamnesis, clinical examination and otoscopy were performed in the right and left sides the ear canal of each dog, in a total 82 samples collected. The otologic samples were collected with sterile swabs placed in a plastic storage tube containing 2 milliliters of sterile phosphate buffer saline (PBS, pH 7.2) and appropriately labeled. Furthermore, otologic swabs were collected for direct yeast examination. All samples were maintained at 4°C in a cooler with ice and forwarded to the Laboratory of Infectious Diseases of Animals (LDIC/DMV/UFRPE).

2.2. Reagents Used for Isolation

For isolation of Mollicutes the samples collected in phosphate buffer saline (PBS, pH 7.2) were cultivated in modified Hayflick's medium. Mollicutes isolates were confirmed by Dienes probe and *Mycoplasma* genus through a digitonin sensitivity test, according to [20].

2.3. Procedures

The Mollicutes isolation procedures consisted in the swabs were homogenized in the transport solution, and 100 uL was relocated to tubes containing 900 uL of modified Hayflick's medium, and diluted up to 10^{-1} at 10^{-5} and spread in 2 mL and agar modified Hayflick's medium. All culture samples sowed were incubated at 37°C in CO₂ incubator for at least 30 days, and blind peals were made for up to 60 days, before disposal of samples. The cultures were checked daily by using a stereomicroscopy (40×) for the presence colonies.

2.4. Statistical Analysis

Associations between Mollicutes and external otitis were analysed by Fisher test, in the Software [21].

3. Results

A total frequency of Mollicutes in the isolation was 32.9% (27/82). 14.8% (4/27) of the dogs positive for Mollicutes were also external otitis and 85.2% (23/27) in dogs without clinical otitis (**Table 1**). The presence of

Table 1. Mollicutes in domestic dogs in the metropolitan region from Recife-PE, Brazil.

Mollicutes	Positive otitis		Negative otitis		Total	
	(AF)	(RF%)	(AF)	(RF%)	(AF)	(RF%)
Positive	4	14.8	23	85.2	27	100
Negative	2	100	0	0	2	100
Total	6		23		29	100

P = 0.036. Absolut frequency (AF), Relative frequency (RF).

fried-egg colonies on solid medium and other appearance variations as raindrops were observed. In the digitonin sensibility test 7.4% (2/27) were positives, confirming presence *Mycoplasma* spp. genus. In the analysis of association of Mollicutes with otitis, this disease influenced negatively the isolation of Mollicutes in the ear canal of dogs, this association was significant (P = 0.036).

4. Discussion

The etiology complex of the canines' otitis has been widely studied in worldwide. Although multiple factors are involved, the species richness of fungi, bacterias and ectoparasites can act as primary factors in clinical otitis in dogs [2]-[6] [22]. Mollicutes are fastidious for bacterial cultivation and few laboratories routinely cultivate these microorganisms, though culture is the most used method for detection of mycoplasmas in dogs [14] [17]. In the present study it was verified high frequency of Mollicutes in the ear canal of healthy dogs as compared to otitis, showing that possibly this bacteria is a component of the normal ear canal microbiota. In this study the dogs with otitis were identified other bacteria and yeasts in the ear canal.

The presence of other bacteria, fungi and yeasts in the ear canal of dogs can compete for nutrients creating undesirable conditions for mycoplasmas growth, since righ requirements and long-term isolation are needed. Several authors [16]-[19] [28]-[30] reported mycoplasmas in different sites of canine body, however in the ear canal there is no references list. The lack of studies may occur as result of greater relevance given to the association of other microorganisms as the main causes of otitis than mycoplasmas. Mycoplasmas have been found as a component of the microbiota of the ear canal in other animals species, such as cattle and goats, therefore it could be source of mycoplasmas for outbreak diseases (pneumonia, otitis, arthritis, mastitis) [23]-[26]. *Mycoplasma cynos*, which is frequently found in dogs respiratory disease, affects the upper respiratory tract. *Mycoplasma edwardii*, *Mycoplasma spumans* and *Mycoplasma maculosum* are usually found in the nasal cavity [11] [12] and *Mycoplasma mucosicanis* found in the oral cavity [15]. Furthermore clinical otitis caused by *Malassezia*, a common yeast found in dogs, acts as a limiting factor to the isolation of Mollicutes, by reason of *Malassezia* genus needs lipid and fatty acids for growth [27]. More studies are necessary to verify the relevance of Mollicutes in the ear canal of dogs and the role these bacteria in the etiopathogeny of otitis and other mycoplasmosis in dogs.

5. Conclusion

This is the first report of members of the Mollicutes class and *Mycoplasma* spp. in the ear canal of dogs however their association with clinical otitis cases remains unclear, and more studies are necessary for proving this relation.

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Conflicts of Interest

The authors declare no conflicts of interest.

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