ABSENCE OF MYCOPLASMA SPP. IN BRONCHOALVEOLAR LAVAGE SAMPLES OF DOGS WITH AIRWAY DISEASE IN A EUROPEAN REFERRAL POPULATION.

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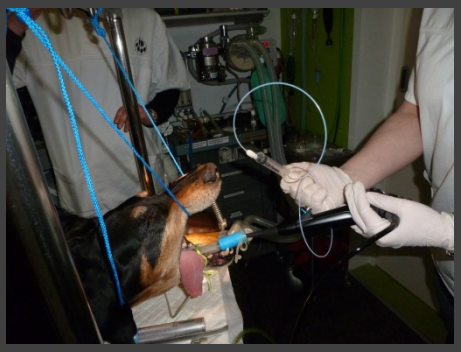
Introduction

Mycoplasma spp. are considered to be part of the normal flora of the nasopharyngeal cavity, however there is still debate whether these agents are primary pathogens in the lower airways. Results of Mycoplasma spp. culture are usually negative from dogs evaluated in our referral center, however, the organisms are known to be fragile in transport and successful culture of Mycoplasma spp. requires special media which is not always used in commercial diagnostic laboratories. Mycoplasma spp. DNA can be amplified by PCR and identified by genetic sequencing and these techniques are now more readily available.

Objectives

The purpose of this study is to report the results of Mycoplasma spp. culture and three Mycoplasma PCR assays on samples obtained from clinically ill dogs.

Material and methods



Bronchoalveolar (BAL) lavage samples were collected prospectively from dogs that were referred for the investigation of respiratory disease. All dogs had a full cardiorespiratory system evaluation (clinical examination, radiographs, fluoroscopy, echocardiography, laryngoscopy and/or bronchoscopy as deemed necessary). None of the dogs had been administered anti-microbial agents with activity against Mycoplasma spp. prior to referral. While under general anesthesia, three 2-5 ml aliquots of sterile saline were passed into an abnormal area of the lung via a bronchoalveolar lavage (BAL) catheter advanced through the bronchoscope channel and recovered by manual aspiration. After collection the BAL fluid was processed for cytologic evaluation and the supernatant cultured for aerobic bacteria and Mycoplasma spp. at a local commercial laboratory. The remainder of the supernatants were frozen at -18 °C within one hour of collection and were shipped to Colorado State University for assay in three previously published PCR assays.

Results

Samples of 21 dogs were obtained from multiple breeds (mainly small), all ages (range 1 - 15y: median 9y), and both sexes (7 F; 14 M). The final diagnoses included eosinophilic bronchopneumopathy (10), chronic bronchitis (4; each with concurrent bronchial collapse), bronchial collapse alone (1), tracheal collapse (1), laryngeal paralysis (1), pulmonary lymphoma (1), infectious bronchopneumonia (1), pharyngitis (1) and a laryngeal polyp (1). All Mycoplasma spp. cultures were negative. While sufficient DNA was detected in each sample using Nanodrop (range: 0.53-4.75; mean 1.58 •g/µl), DNA of Mycoplasma spp. was not amplified from any sample with any of the three PCR assays.

N°	Breed	Mycoplasmae PCR			Nanodrop
		Johnson-Myco	Chalker-M.canis	Chalker-M.cynos	DNA quant (ng/ul)
1	CV	0	0	0	0,575
2	Whippet	0	0	0	1,395
3	Beagle	0	0	0	1,57
4	Bichon	0	0	0	1,14
5	Corgi	0	0	0	1,56
6	CV	0	0	0	1,44
7	Spitz	0	0	0	1,64
8	Chihuahua	0	0	0	1,55
9	JR	0	0	0	0,8
10	Cocker	0	0	0	0,96
11	ShiTzu	0	0	0	1,9
12	CV	0	0	0	1,39
13	CV	0	0	0	0,85
14	CV	0	0	0	4,75
15	Maltezer	0	0	0	0,53
16	ShiTzu	0	0	0	2,52
17	CV	0	0	0	2,145
18	Pinsher	0	0	0	2,19
19	Border Collie	0	0	0	1,59
20	ShiTzu	0	0	0	1,55
21	Dachshund	0	0	0	1,05

Conclusion

In conclusion, failure to grow Mycoplasma spp. or amplify Mycoplasma spp. DNA from these patients suggests that the organism was not involved in the pathophysiology of disease.