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011A.1 MOLECULAR EVIDENCE OF POLYMORPHISM IN PNEUMOCYSTIS FROM WILD MICE AND SHREWS IN THE EASTERN ALPS, ITALY

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INTRODUCTION:

Pneumocystis organisms are considered as major opportunistic fungal pathogens that infect humans and a wide range of other mammalian host species. The genus comprises uncultured, host-specific parasites adapted to the alveolar epithelial cells type I where they may proliferate provoking severe pneumonitis. Thus far, five species have been described: *P. carinii* and *P. wakefieldiae* in brown rats, *P. jirovecii* in humans, *P. murina* in common mice and *P. oryctolagi* in rabbits.

In the present study, we detected and characterised *Pneumocystis* organisms in wild mice and shrews from eastern Alps. Our objective was to assess whether *Pneumocystis* organisms could be used as phylogeographic markers for these mammalian species.

MATERIALS AND METHODS:

A total of 96 lung tissue samples from wild rodents and insectivores were analyzed. Specimens of *Apodemus flavicollis* (n=30), *Apodemus* sp (n=1), *Myodes glareolus* (n=3), *Sorex arthorini* (n=61), and *Sorex* sp (n=1) were captured as part of an large collaborative European project (EDENext) by Fondazione Edmund Mach. After DNA extraction, samples were analyzed by nested PCR, using primers for the gene encoding the mitochondrial large subunit rRNA (mtLSU rRNA) (Derouiche et al. 2009) and sequenced. The mtLSU sequences were aligned with already known *Pneumocystis* sequences using Clustal X (version 1.63b) and a phylogenetic analysis was performed using Neighbor Joining integrated in Mega 4 software (version 4.0).

RESULTS:

Pneumocystis DNA was detected in 58 of 96 samples (60%). Lung tissues from *Apodemus flavicollis* (12/30; 40%), *Sorex arthorini* (44/61; 72%) and *Myodes glareolus* (2/3; 67%) were positive. The mtLSU sequences showed marked polymorphism with seven *Pneumocystis* sequence types representing three distinct major clades according to the three animal species. Among *Sorex* genus all sequences presented a similarity of 100%, whereas six different sequence types were obtained for the genera *Apodemus* (n=4) and *Myodes* (n=2).

CONCLUSIONS:

These preliminary observations are in agreement with previous findings, which reported similar prevalence of *Pneumocystis* in *Apodemus* and *Myodes* animals as well as the highest prevalence in *Sorex* spp., when compared with other wild mammals (Laakkonen et al., 1995; Can J Zool. 73:961-966). The genetic polymorphism in *Apodemus* and *Myodes* has already been described and seem to be a general tendency for rodent-derived *Pneumocystis*. The unique sequence type detected among *Sorex* shrews from the eastern Alps suggests that *Pneumocystis* from insectivores display a lower level of genetic polymorphism.

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Keywords:

Pneumocystis, wild mice, Molecular polymorphism

011A.2 SURVEY ON YEASTS IN MUCCOSAE OF DOGS WITH AND WITHOUT WEAKENING PATHOLOGIES

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INTRODUCTION:

In human medicine many studies point out as yeasts, commensals on mucosae, can become pathogens in course of immunodeficiency, chemotherapy, corticosteroid therapy, endocrinopathies (Bulacio et al. 2012, J. Mycol. Med., 22: 348-353). Such risk factors were also suggested in Veterinary Medicine but, so far, only few studies about this topic were made (Tampieri et al. 2008, Parasitologia, 50: 95; Bieganski et al., 2012, 18th congress (SHAM), p.177). In this paper, the yeast flora of oral, conjunctiva and rectal mucosae of dogs was examined, in relation to presence ("sick dogs") or absence ("healthy dogs") of predisposing factors such as endocrinopathy, neoplasia, corticosteroid therapy or other weakening pathologies.

MATERIALS AND METHODS:

One hundred eight dogs (61 "healthy" and 47 "sick") were examined; from each dog 4 swabs from oral, conjunctival and rectal mucosae were collected and inoculated in duplicate on Sabouraud Dextrose Agar (BBD) with 0.05g/ chloramphenicol (Sigma). The plates were incubated both at 25°C and 37°C ad observed daily for 7 days. The yeasts isolated were identified microscopically for *Mucosae* *padhydermatis* and with Api 20C AUX (Biomerieux Italia S.p.a, Firenze, Italy) and Dalmu plates on Yeast Morphology Agar DIFCO (Becton Dickinson Italia S.p.a, Milano, Italy) for the other yeasts.

RESULTS:

Yeasts were found in 83 dogs out of 108 examined (76.8%). *M. pachydermatis* was isolated in at least one sample from 78/108 dogs (73.2%), especially in rectal mucosae where also the major number of yeast species was present. Conversely, lowest yeasts isolation was from conjunctival swabs. The other yeasts isolated were: *Rhodotorula* spp (8.34%), *Candida albicans* (3.7%), *C. glabrata* (2.78%), *Cryptococcus laurentii* (1.85%), *C. guilliermondii*, *C. parapsilosis*, *C. boidinii*, *Saccharomyces cerevisiae* and *Cr. albidus* (0.92%). *M. pachydermatis* was found more frequently in healthy dogs (83.6%) compared to sick dogs (57.4%) (X² = 6.18, p<0.05). On the contrary, other yeasts were found more frequently in sick dogs (15.22%) than in healthy dogs (6.2%) (Fisher exact p<0.05). In particular, *C. albicans*, isolated only in 4 sick dogs (8.7%), was not found in healthy ones (Fisher exact p<0.05). In one dog with parvovirus, *C. albicans* was isolated from an ulcer of oral mucosa that resolved without specific treatment after healing.

CONCLUSIONS:

M. pachydermatis was the yeast most frequently present on dogs mucosae, according to Tampieri et al. (2008 l.c.). Brito et al. (2009, Vet. J., 182: 320-326) and Santh et al. (2013, Braz. J. microbiol., 44: 139-143) and can be considered an usual component of mucosal microflora of dog. The isolation of *S. cerevisiae* could be explained by the administration of probiotics: *C. boidinii*, *C. parapsilosis*, *Cr. laurentii*, *Cr. albidus* and *Rhodotorula* spp. isolated, with low number of colonies, only at 25°C, could be considered as environmental contaminants and not colonizer, contrary to *C. glabrata* and, especially, *C. albicans* that were isolated both at 25°C and 37°C, only in sick dogs and in high number of colonies. From these observations we can suppose that may be a real colonization of mucous membranes. Unlike what happens in humans, where *C. albicans* is a frequent commensal in the mucosa of healthy subjects, in the dog the finding of such yeast is occasional and could be a proof of organic imbalance.

Keywords:

Yeast, Mucosae, Dog