Bs En 12449 Pdf 17

A different Sarcocystidae was detected in the two contrasting environments of Tioman Island. Water samples captured between 2014 and 2015 from the eastern (PCI) and northern (SSP/SMP) region of the island, respectively, were analysed using the 28S R7F28S R8 Deg R primer set. A total of 16 of the 32 water samples, as well as two out of the 25 soil samples, were positive for Sarcocystidae based on the presence of the 28S rRNA gene. Analysis by amplicon sequencing of the 28S gene variable region revealed seven distinct Sarcocystidae sequences. The sequence generated from the 18S rRNA gene V9 hypervariable region detected only two Sarcocystidae species, S. singaporensis and S. nesbitti, in the two contrasting regions and also in both soil samples. Each of the sequences that were detected in this study was named according to the genus, the host sample it was obtained from, the region from where the sample was acquired, the sampling date and the country and publication reference. Severe acute sarcocystosis

occurred in summer 2012 among visitors of the Tioman Island in south Malaysia. The confirmation of the causal agent was attempted only by the 18S rRNA gene sequencing, since the development of an in-house developed method of PCR-RFLP on the 544 bp of the 28S rRNA was not yet successful. Additionally, for the early identification of species, the nomenclature of the 28S rRNA gene sequences was pre-determined. In this study, a new primer set (28S R7F28S R8 Deg R) was designed and used to amplify the partial 28S rRNA gene in 11 cases, in which only a single species was found. The detection rate was 100% (11/11). Sequences were named by GenBank accession number and the species, region of origin, sampling time and sampling reference. Of the 11 new sequences generated, 10 matched a sequence detected in S. singaporensis (97.7%) and one matched a sequence detected in S. nesbitti (90.9%). The latter sequence was also obtained from soil and was named Sarcocystis sp. YLL-2013.

Bs En 12449 Pdf 17

DNA extracts and filtrates were further tested for their ability to amplify a PCR reaction using two different primer pairs, 18s R5F (5'-TTAACACGGGAAGCAGCAG-3') and 18s R6R (5'-CCTCCCTCGCGCCATCAC-3') and 28S R9R (5'-TGCACCTCTTTGGGCAGC-3') and 28S R10F (5'-CATCCCCATCACCTACGAC-3') primers (Lane & Ogden 1991) to obtain DNA fragment lengths of about 1530 and 1110 bp, respectively. PCR reactions for both primer pairs yielded DNA fragments from both filtrates of

environmental samples (presumptively positive samples) and the control samples. No PCR product was detected from filtrates of negative samples. There was no difference in the DNA fragment sizes among environmental samples (presumptively positive) and the control samples (data not shown). These findings suggest that both primer pairs were able to amplify Sarcocystidae DNA fragments from water samples, regardless of their origin or filtration or centrifugation. In addition, filtrates were potentially useful in detecting Sarcocystidae eggs. Unfortunately, neither filtrates nor PCR products could be sequenced due to insufficient amount of DNA. Moreover, sample extracted from all the filtrates failed to amplify PCR products with one primer pair, 18S R5F and 18S R6R, although samples were positive with the other primer pair, 28S R9R and 28S R10F (data not shown). These results suggest that either sample preparations for extraction and amplification were incorrect or there were other non-Sarcocystidae parasitic organisms that might have been present in the water. Conventional PCR amplifications of Sarcocystidae DNA fragments of the filtrates was performed with two primer pairs, namely, 28S R4F (5'-TGCACTCCGTGTTTCAAGAC-3') and 28S R5R (5'-CATGCAGCTCACGTCACTA-3'), and 16S R1F (5'-CCCTTATGCTTAGATCAG-3') and 28S R6F (5'-CGTGACTGATGCTTGTATG-3'), targeting the small subunit ribosomal RNA genes (SSU rDNA). Both the conventional PCR products contained DNA fragments of about 590 bp. Amplification of DNA fragments of both primer pairs showed variable results, ranging from

positive to negative. Of all the replicates of the filtrates from various locations, samples from L2 and L3 were positive with one primer pair, 28S R4F and 28S R5R, while samples from L4, L5, L6 and L7 were positive with the other primer pair, 16S R1F and 28S R6F. The overall amplification of Sarcocystidae DNA fragments, based on the number of positive replicates, suggested that sequencing the positive samples would be more useful in identifying sequences of Sarcocystidae to the species level than PCR amplifications of filtrates. 5ec8ef588b