Detection of Cryptosporidium sp. in stool, feces, and drinking water samples in Austria

Cryptosporidiosis (Cryptosporidium spp.) are obligate parasitic protozoa of different vertebrates including man; infections cause severe diarrhea and may lead to death of immunocompromised hosts, man as well as animals. Nevertheless, although the waterborne nature of several human cases is well known and documented, the accurate infection routes and the reservoirs are still unknown to a great extent. The reason is a considerable uncertainty about the host specificity of the genus Cryptosporidium and the species delimitation. Some Cryptosporidium strains (Cryptosporidium parvum “human”) are fatal parasites of AIDS-patients, whereas others (C. parvum “cat”) may cause death in cats but only moderate disease in man. On the other hand, pet reptiles have been found to be infected by C. parvum (unknown form) and by C. serpents simultaneously - species causing death in snakes - and, additionally, they may infect C. muris-cows.

Determining Cryptosporidium species and strains is most efficiently done by DNA-hyping and DNA-amplification. The polymerase chain reactions, which may be used simultaneously as diagnostic tools in samples of various nature. By applying a combination of diagnostic tests, isolation procedures and typing techniques, epidemiological data are created about the occurrence of these parasites within an area and within host populations. Pet animals, exotic, too, one may act as vectors or as reservoirs for human infections, and water supplies may spread the parasites into large susceptible host populations.

In Austria, a state in the center of Europe, Cryptosporidiosis is a known parasitic disease. Yet, no facts exist about the distribution of these parasites and about the frequency of cryptosporidiosis in cattle and pets. Since 1984, cryptosporidiosis is a fatal abdominal disease in Austrian AIDS-patients and since a few years such infections are accidentally also diagnosed in immunocompetent Austrians, especially in young persons. A few years ago a project on hygiene in vivaria was initialized, including the detection of Cryptosporidium in reptiles and amphibians, mostly animals living in a zoo. Some fecal samples were also taken from free-living lizards during excursions to nature reserve areas. We tried to compare the infection rate with Cryptosporidium sp. of captive reptiles with the one of reptiles far away from any human influence to assess the importance of exotic pet animals as vectors of zoonotic human disease.

Moreover, sediment samples of drinking water and of water for human consumption were collected by flat bed filtration mostly in the eastern parts of Austria. In search of enterobacteria and of Cryptosporidium the fecal source of a conata-miniation should be made plausible and the distribution pattern of these Enteromicrobes should be characterized.

Previously several detection tests were rated for efficiency and some were organized in a modular manner to meet the different requirements during such a multi-artistic study.

The water samples (150 l each) were filtered through flat bed filters (cellulose acetate, 1.2 µm pore size, Sarstos Geosynth, Vienna, Austria), the sediments were collected by filter lysis and centrifugation. Human stool samples and feces of exotic pet animals were dispersed into physiological saline, respectively. One of three parts of each sample was applied to slides, air-dried, and colored in a modified Zehl-Neelsen staining. From one part of the samples, C. parvum-cysts were isolated by an immunomagnetic procedure according to the manufacturer’s instructions (DINAG GmbH, Hamburg, Germany). DNA was extracted from the last part of the samples by adsorption technique (Qiagen GmbH, Hilden, Germany) and amplified in a minor modified polymerase chain reaction for detection of the 18S gene of the 18S rRNA of Cryptosporidium sp. according to Morgan et al. 1997. The detection of amplified DNA was done by flat bed electrophoresis and silver staining. The amplifications of the different species were recognized by their size.

Concerning the detection of oocysts of Cryptosporidium sp. in samples of various nature, the most efficient method is the modified Zehl-Neelsen staining. Only minor modifications of this universal technique are known, mostly due to confusion with the fresh water algae Cocyclosis sp. Nevertheless, especially in environmental samples a differentiation of the Cryptosporidium species is necessary in most cases. DNA amplification techniques are far more sensitive methods than microscopic examination techniques. Moreover, a comparison of DNA-sequence data and contamination with poorly defined or still undescribed Cryptosporidium species are overlooked.

Until now, nobody exists in Austria about the frequency of Cryptosporidium contamination of environmental samples. But there is a minor hint regarding a decrease in the number of human infections since 1990; 92.6% of the Austrian HIV-1-infected persons shed oocysts at that time, now we found 4.3%, maybe due to a lower number of AIDS-patients. Exotic pet animals, chimpanzees as well as reptiles, sometimes shed Cryptosporidium oocysts, even C. parvum-cysts infective for man. The source of these infections or contaminations is still unknown, anthropogenic origin is likely. Immunodeficient person should be aware of the health hazard starting out from keeping exotic pet animals.

At present a procedure is in trial for a recognition of most of the Cryptosporidium species in various samples. This procedure is based on a modular arrangement of independent processing steps. The core assumption is a gene amplification technique after a purification step.

Literature: