Cryptosporidium (Cryptosporidium spp.) is a zoonotic parasitic protozoan of different vertebrates including man: infections cause severe diarrhea and may lead to death in immunocompromised hosts, man as well as animals. Neurotropic infections are widespread among several human cases is well known and documented, the accurate infection routes and the reservoirs are still unknown to a great extend. 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 calves but only moderate disease in man. On the other hand, some reptiles have been found to be infected by C. parvum (unknown form) and by C. serpentis simultaneously - a species causing disease in snakes. Additionally, reptiles may shed C. multi-ocytoides.

Determining Cryptosporidium species or strains is most efficiently done by DNA-typing after DNA amplification by polymerase chain reactions. PCR may simultaneously be used as a diagnostic tool 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 a host population. Pet animals, especially reptiles and, maybe, amphibians too, may act as vectors and/or as reservoirs for human cryptosporidiosis, and water supplies may spread the parasites into large susceptible host populations.

In Austria, more precisely in Lower Austria, Cryptosporidium are long known cattle parasites. Yet, no detailed knowledge existed about the local distribution of these parasites and about the frequency of cryptosporidiosis in cattle and other animals. Since 1984 Cryptosporidium is diagnosed as a zoonotic disease in Austrian AIDS-patients and since a few years such infections are accidentally also found in immunocompetent Austrians, especially in young persons.

Thus, numerous stool samples were routinely tested for Cryptosporidium and in some cases of occult shedding the parasite species was determined. A few years ago, a project on hygiene in vivaria was initiated, including the detection of Cryptosporidium in reptiles and amphibians, mostly animals living in a zoo. Some feces samples were taken from feed containers as standards during excursions to nature reserve areas in Southeastern Austria and Slovenia. In this retrospective study based on a self-developed, modified accurate Cryptosporidium detection system we compare the infection rates of captured reptiles and amphibians with the ones of reptiles for away from any human influence. Moreover, recent samples of drinking water and of water for human consumption were collected by flat bed filtration methods in Lower Austria. In spitting of enterobacteria and of Cryptosporidium the fecal source of a contamination should be made plausible and the distribution pattern of these parasites should be made discernable.

The water samples (150 l each) were filtered through flat bed filters (cellulose acetate, 1.2 μm pore size, Sarstedt Gesmibii, Vienna, Austria). The sediments were collected by filter disks and centrifugation. Human stool samples and feces of reptiles and amphibians were dispersed into physiological saline. One of three parts of each sample was applied to slides, dried, and stained by a modified Ziehl-Neelsen procedure. From one part the C. parvum-oocytes were isolated by an immunomagnetic procedure according to the manufacturer’s instructions (DNA LiC GmbH, Hamburg, Germany). DNA was extracted from the last part of the sample 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 in samples of miscellaneous nature, the most efficient method is a modified Ziehl-Neelsen staining. Only minor pitfalls of this universal technique are known, mostly due to a corrosion with the freshwater orga Oncorhynchus sp. But in practice, in almost all cases of a Cryptosporidium detection accurate differentiation of the parasite species is required. DNA amplification techniques as more sensitive and powerful methods than microscopic examination techniques, but due to our limited knowledge of DNA sequence data infections or contaminations with different species can be described. Cryptosporidium species may be overlooked.

Infections with diarrhea are frequently infected with Cryptosporidium (4.3%), whereas immunocompetent persons without intestinal symptoms rarely harbour these parasites (0.5%). Thus, in Austria the occurrence of an endemic disease caused by Cryptosporidium is unlikely. Captured amphibians and reptiles sometimes excrete Cryptosporidium oocysts, in most cases C. serpentis but occasionally also C. parvum. The sources of these infections are still unknown, an influence of the human keeping activity is very likely. Cryptosporidium are parasites of immunocompromised, weasen herptiles, as free living ones seem to be infected rarely, if ever. Water for human consumption may act as carrier of Cryptosporidium oocysts. As we could not detect any correlation between the appearance of oocysts and fecal contamination indicator bacteria, and standard water distinction measurements are ineffective to a large extent to demand parasite-free raw water sources.

Literature: