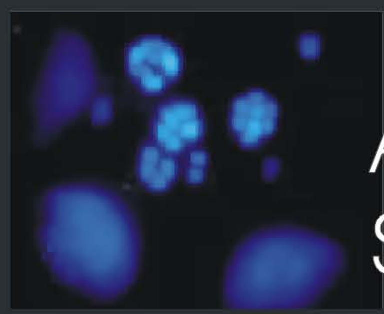

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Cryptosporidium spp. in faecal and water samples in Austria

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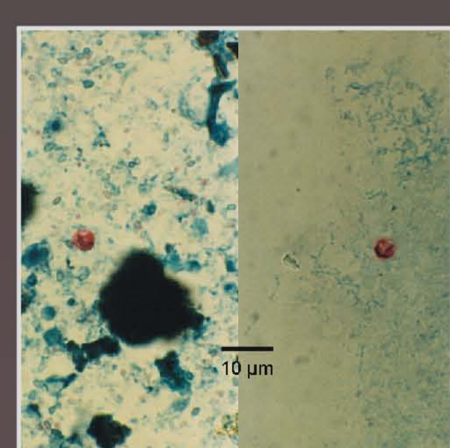
Cryptosporida (*Cryptosporidium spp.*) are obligatory parasitic protozoa of different vertebrates including man; infections cause severe diarrhea and may lead to death of immunocompromised hosts, man as well as animals. Nevertheless, although the waterborne nature of several human cases is well known and documented, the accurate infection routes and the reservoirs are still unknown to a great extend. The reason is a considerable uncertainty about the host specificity of the genus Crytosporidium and the species delimitation: Some Cryptosporidium strains (*Crytosporidium parvum* "human") are fatal parasites of AIDS-patients, whereas others (*C. parvum* "calf") may cause death in calves but only moderate diarrhea in man. On the other hand, pet reptiles have been found to be infected by *C. parvum* (unknown form) and by *C. serpentis* simultaneously - a species causing death in snakes - and, additionally, reptiles may shed *C. muris*-oocysts.

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 out the parasites into large susceptible host populations.

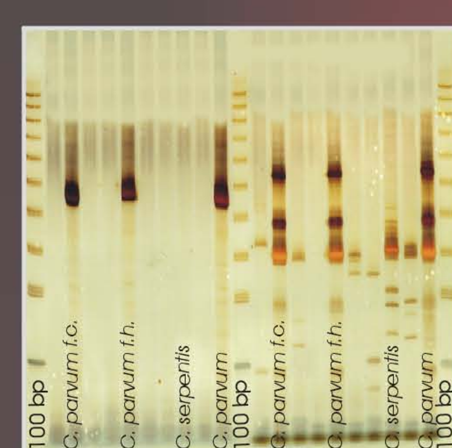
In Austria, more precisely in Lower Austria, Cryptosporidia are long known cattle parasites. Yet, no reliable knowledge exists about the local distribution of these parasites and about the frequency of cryptosporidiosis in cattle and other animals. Since 1984 cryptosporidiosis is diagnosed as fatal abdominal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

Thus, numerous stool samples were routinely tested for Cryptosporidium and in some cases of oocyst shedding the parasite species was determined. A few years ago a project on hygiene in vivaria was initialized, including the detection of Cryptosporidia in reptiles and amphibians, mostly animals living in a zoo. Some feces samples were also taken from free-living lizards during excursions to nature reserve areas in Southeastern Austria and Slovenia. In this retrospective study based on a self-developed, modularly arranged Cryptosporidium detection system we compare the infection rates of captured reptiles and amphibians with the ones of reptiles far away from any human influence. 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 Cryptosporidia the fecal source of a contamination should be made plausible and the distribution pattern of these parasites should be made discernable.

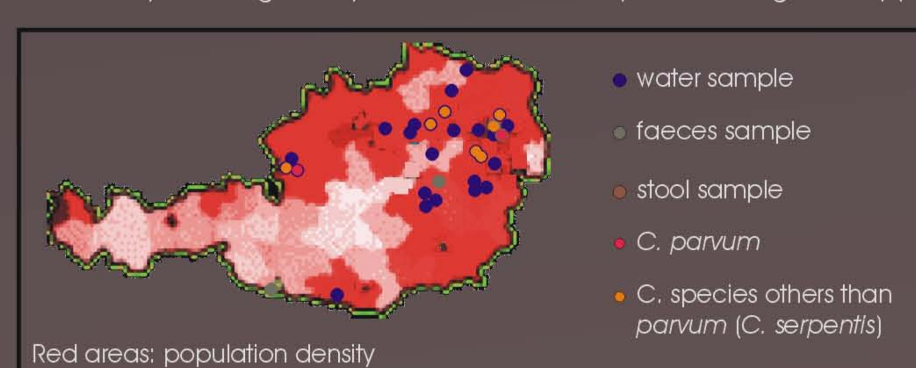
The water samples (150 l each) were filtered through flat bed filters (cellulose acetate, 1.2 μm pore size, Sartorius GesmbH, Vienna, Austria), the sediments were collected by filter lysis and centrifugation. Human stool samples and feces of reptiles and amphibians were dispersed into physiological saline. 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*-oocysts were isolated by an immunomagnetic procedure according to the manufacturer`s instructions (DYNAL 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 ITS1 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 amplificons of the different species were recognized by their size.



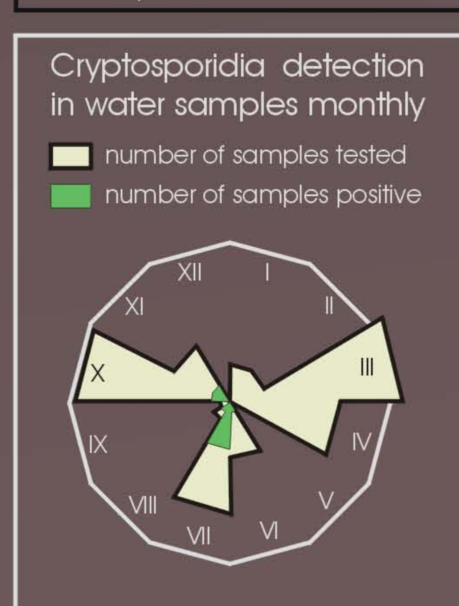
Modified Ziehl-Neelsen staining: left: *C. serpentis* right: *C. parvum*

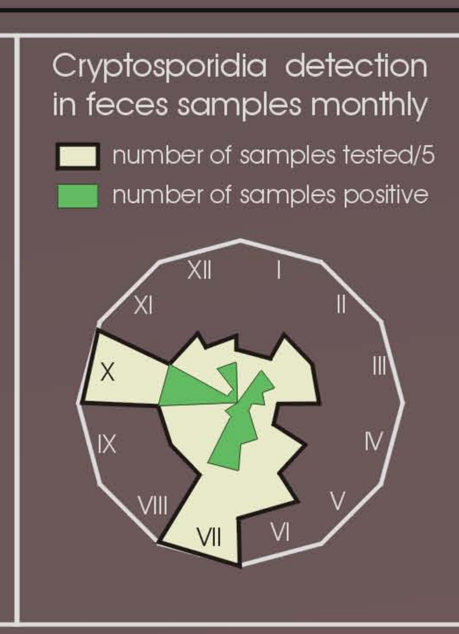


Polymerase - chain - reaction: left: *C. parvum* right: C. spp.



sample	n=	pos	%	C. species (in %)
stool HIV-pos	639	21	3.3	undetermined
human stool (1)	1066	5	0.5	undetermined
diarrhoea stool	437	19	4,3	C. parvum (100)
feces of captured amphibians & reptiles	450	21	4.6	C. serpentis (24) C. parvum (14) unknown (62)
feces of free living amphibians & reptiles	52	0	0	
drinking water	109	11	10	C. parvum (18) unknown (82)
water for human consumption	6	0	0	





Cryptosporida oocysts could be detected in water samples, in human stool, and in feces of captured amphibians and reptiles. Testing statistical significance no correlation was found between oocyst shedding and the host`s sex, age, the type of diet, the type of environment, and the quantity of cattle breeding in Austrian regions, respectively.

Concerning the detection of oocysts of *Cryptosporidium sp.* 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 confusion with the freshwater algae *Oocystis sp.* But in practice, in almost all cases of a *Cryptosporidium* detection accurate differentiation of the parasite species is required. DNA amplification techniques are far more sensitive and powerful methods than microscopic examination techniques, but due to our limited knowledge of DNA-sequence data infections or contaminations with poorly defined or still undescribed *Cryptosporidium* species may be overlooked.

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

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