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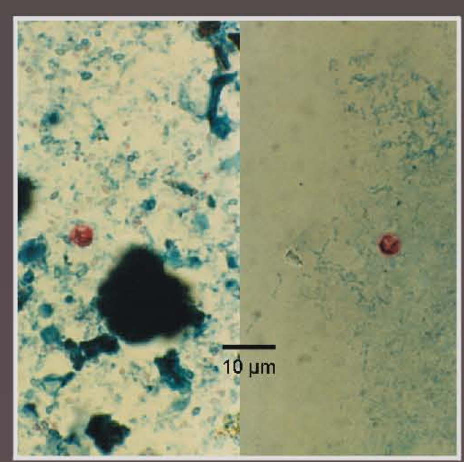
Detection of Cryptosporidium sp. in stool, feces, and drinking water samples in Austria

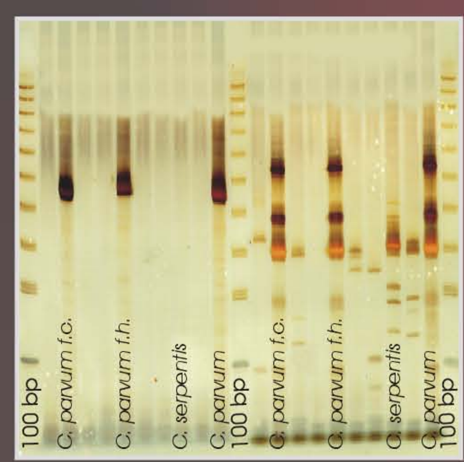
Cryptosporida (Cryptosporidium spp.) are obligatory parasitic protozoa of different vertebrates including man; infections cause severe diarrhea and may lead to death of immunocompromised hosts, man as well as animals. Nevertheless, although the waterborne nature of several human cases is well known and 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, they may shed *C. muris*-oocysts.
Determining Cryptosporidium species and strains is most efficiently done by DNA-typing after DNA amplification by 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 one too, may act as vectors or as reservoirs for human infections, and water supplies may spread out the parasites into large susceptible host populations.

In Austria, a state in the center of Europe, Cryptosporidia are long known parasites of cattle. Yet, no facts exist about the distribution of these parasites and about the frequency of cryptosporidiosis in cattle and pets. Since 1984 cryptosporidiosis is diagnosed as 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 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. We tried to compare the infection rate with Cryptosporidium sp. of captured 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 diseases.
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 conta-mination should be made plausible and the distribution pattern of these Eimerian parasites should be made discernable.
Previously several detection tests were rated for efficiency and some were organized in a modular manner to meet the different requirements uprising during such a multifarious study.

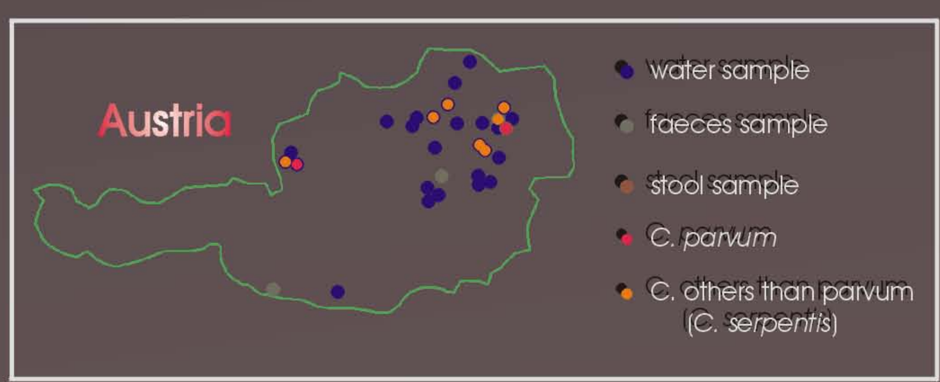
The water samples (150 l each) were filtered through flat bed filters (cellulose acetate, 1.2 µm pore size, Sartorius GesmbH, Vienna, Austria), the sediments were collected by filter lysis and centrifugation. Human stool samples and feces of exotic pet animals were dispersed into physiological saline, respectively. One of three parts of each sample was applied to slides, dried, and colored in a modified Ziehl-Neelsen staining. 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.



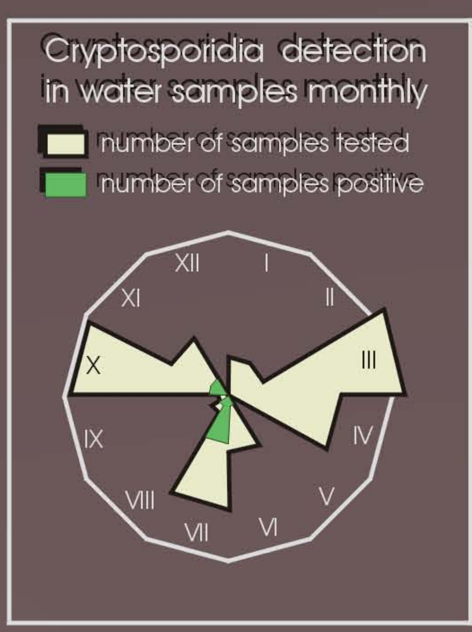
Modified Ziehl-Neelsen staining:
left: *C. serpentis* right: *C. parvum*

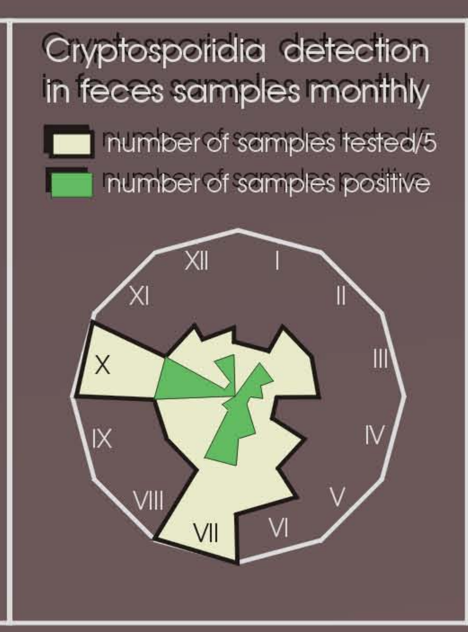


Polymerase - chain - reaction:
left: *C. parvum* right: C. spp.



sample	n =	found positive n =	positive in %	Cryptosporidium species (in %)
human stool	437	19	4.3	*C. parvum* (100)
feces of zoo living herptofauna	450	21	4.6	*C. parvum* (14) *C. serpentis* (24) C. undefined (62)
feces of free -living reptiles	29	0	0	-
drinking water	109	11	10	*C. parvum* (18) C. undefined (82)
water of human use	6	0	0	-





Cryptosporida oocysts were detected in water samples, human stool, and feces of exotic pet animals. Amphibians were infected as well as reptiles, especially snakes. No correlations were found between Cryptosporidium infections and the type of diet, the type of environment, and the quantity of cattle breeding in Austrian regions.

Concerning the detection of oocysts of Cryptosporidium sp. in samples of various nature, the most efficient method is the modified Ziehl-Neelsen staining. Only minor pitfalls of these universal technique are known, mostly due to confusion with the freshwater algae Oocystis 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, but due to our limited knowledge of DNA-sequence data infection or contamination with poorly defined or still undescribed Cryptosporidium species are overlooked.
Until now, no data exist 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: 9.2% of the Austrian HIV1-infected persons shed oocysts at that time, now we found 4.3%, maybe due to a lower number of AIDS-patients.
Exotic pet animals, amphibians as well as reptiles, sometimes shed Cryptosporidium oocysts, even *C. parvum*-oocysts infective for man. The source of these infections or contaminations is still unknown, anthropogenic origin is likely. Immunodeficient persons 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 known Cryptosporidium species in various samples. This procedure is based on a modular arrangement of independent processing steps - the core com-ponent is a gene amplification technique after a purification step.

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