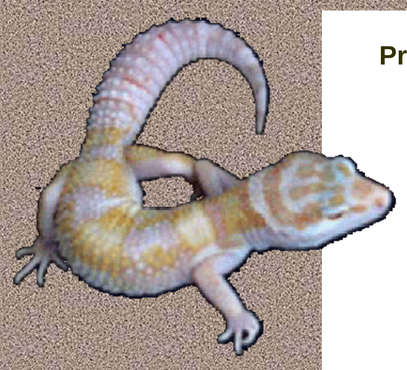
44th ÖGTP Meeting
Graz, November 18th – 20th, 2010
Vet3

Hygiene-relevant characteristics of ordinary intestinal parasites of pet Leopard geckos

Andreas R. Hassl

Department of Specific Prophylaxis and Tropical Medicine
Center for Pathophysiology, Infectiology & Immunology
Medical University Vienna

Facilitated by: Micro-Biology Consult Dr. Andreas Hassl



Pr. Background information: In Central European households Leopard geckos (*Eublepharis macularius*, Blyth 1854) are common and very popular exotic pets. The reasons for this spread are the simple handling of these small insectivore nocturnal ground-dwellers, naturally found in the deserts of Southern Central Asia, their appearance as breathtaking colourful “morphs”, and the husbandry restrictions by the recently implemented Animal Protection Legislation. In about 90 000 Austrian households pet reptiles are kept (1). We estimate, that about 50% of all desert vivaria, the most common type of terraria in the leisure sphere, are inhabited by some Leopard geckos, in more than half of the cases care is taken for less than 5 animals per house-hold. Together with the numerous Leopard geckos in commercial breeding farms and the Transeuropean shipping of reptiles intended for pets a colossal, wide-ranging reservoir for pet reptile associated infectious microorganisms is artificially created.

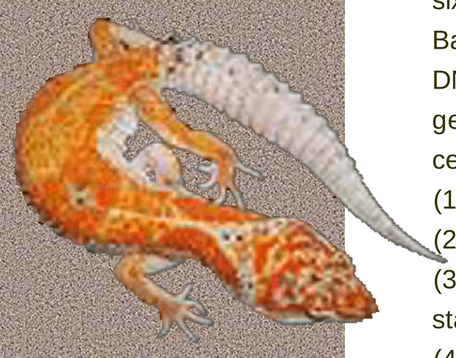
As no longer free living Leopard geckos are taken from the wildlife for initializing pet animal husbandry, the species spectrum of microorganisms parasitising Leopard geckos living in captivity for many generations has switched. Only reptilian parasites able to survive within the artificial, minimal habitat of a terrarium are represented in the current microorganism spectrum, supplemented with ubiquitous, host-unspecific, frequently opportunistic and man-associated infectious microorganisms (2). We anticipate a very limited number of parasite species able to infect pet Leopard geckos in captivity, characterised by

- a simple, mostly monoxenous life cycle
- a high infectivity and a low pathogenicity
- a host immune status depending opportunity
- and a man-boosted transmission route.

Indeed there is a limited number of studies on some specific parasitic protozoa in pet or stock reptiles, especially Leopard geckos (3,4,5) and a very limited number of investigations of parasitic infections in free-living geckos (6,7), but there are no data available reflecting the particular selection criteria affecting successful parasites in artificial habitats.

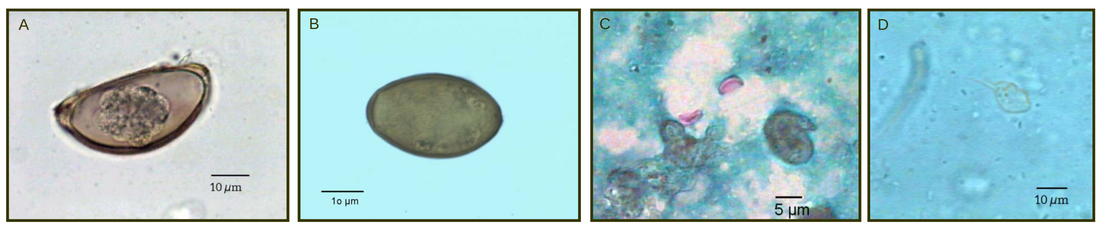
1. Materials and Methods: Between 2006 and 2009 250 fecal samples of 332 Leopard geckos (*Eublepharis macularius*), 32% males and 60% younger than six month, from 32 pet stocks located in Vienna, Lower Austria, Thuringia, and Bavaria were tested for their contents of visible parasite stages and parasite DNA. All fecal samples were taken from reptiles bred in captivity for several generations and reared by stock owners organized in internet fora. The test procedures applied to all fecal samples were

(1) a visual inspection of the fecal sample,
(2) a microscopical examination of an aqueous suspension of the feces,
(3) a microscopical examination of a fixed layer after a modified Ziehl-Neelsen staining, and
(4) two simple Polymerase-Chain-Reactions for the detection of Cryptosporidium-, and Entamoeba-DNA, according to Morgan et al. (8) and Verweij et al. (9), respectively.



The results of the PCRs served as marker for a verification of the specificity and the sensitivity of the visual tests and as a corrective of these test results.

2. Results: In Central Europe only a very limited number of intestinal parasite species exists infecting pet Leopard geckos: An ubiquitous Oxyura (A), most probably a Parapharyngodon or, less likely, a Pharyngodon species, a Nyctotherus species (B), that is a ciliated protozoa with ambiguous parasitic features, some more or less opportunistic Cryptosporidia species (C), mostly *C. varanii*, and Monocercomonas sp. (D), most probably *M. colubrorum*, which may turn out to be a genuine insect parasite. In a single case a probably protracted *Entamoeba invadens* infection was found, but these data were not processed in detail.



geckos n = 332	Parapharyngodon sp.	Nyctotherus sp.	*Cryptosporidium varanii*	Monocercomonas sp.	*Entamoeba invadens*
pos samples n =	94	7	28	5	1
% pos of all samples	37,6	2,8	11,2	2,0	0,4
% pos of all geckos	39,8	2,1	8,4	1,5	0,3

male geckos n = 31	Parapharyngodon sp.	Nyctotherus sp.	*Cryptosporidium varanii*	Monocercomonas sp.
pos sample n=	8	1	2	1
% pos of all samples	26,7	3,3	6,7	3,3
% pos of all male geckos	25,8	3,2	6,5	3,2
female geckos n = 67				
pos sample n=	12	2	0	6
% pos of all samples	24,5	4,1	0,0	12,2
% pos of all female geckos	23,9	3,0	0,0	11,9
p =			0,034	**0,0033**

gecko < 6 month n = 30	Parapharyngodon sp.	Nyctotherus sp.
pos sample n =	3	0
% pos of all samples	18,8	0,0
% pos of all young geckos	30,0	0,0
gecko > 6 month n = 21		
pos sample n =	6	1
% pos of all samples	50,0	8,3
% pos of all mature geckos	38,1	4,8

stock owner n =	geckos n =	Parapharyngodon n =	Parapharyngodon %	Cryptosporidium n =	Cryptosporidium %
1	43	13	30,2		
1	32	14	43,8	1	3,1
3	30	13	27,8		
1	26	15	57,7	3	11,5
1	22	7	31,8		
1	17	8	47,1	5	29,4
1	15	12	80,0	6	40,0
2	11	7	59,1	1	9,1
1	10	5	50,0	3	30,0
1	8	3	37,5	4	50,0
19	max 5; Ø 2,5	15	36,2	5	10,6

3. Insights: Recent changes in the human behaviour and the social structures especially in the urban environments open up new ecological niches and artificial habitats for synanthropic animals, some of them - like the Leopard gecko - on their way to domestication, and their parasites. Close gathering and frequent cohabitation of man and pet reptiles cause such habitat changes which increase the transmission rates of some pathogens, create new infection routes, and foster the emergence or uprising of zoonotic diseases.

Due to modern transportation and communication facilities pet reptile keepers are a close community and they group their pets, the Leopard geckos, into an animal pool and thereby configure an effectual reservoir for some highly specialised infectious microorganisms. Intestinal parasites are typically pathogens with a straightforward, highly effective transmission route, especially if it is combined with live stages that are resistant to most environmental impacts, like (Oxyura) eggs or (Cryptosporidia) oocysts. All parasites detected are ordinary, ubiquitous, and more or less opportunistic infectious microorganisms with a low pathogenicity and a high infectiousness, except the possibly protracted Entamoeba.

The geckos have a moderate or low and species-poor parasite burden in the majority of cases, a status enforced by long-time pet keeping and farm breeding. Distinctive features of our findings are the high infection rate even of baby gekkos with Oxyura - up to 40% - and the statistically significant but unexplainable difference in Monocercomonas-infections between male and female geckos.

With regard to a forthcoming establishment of a cost-effective prognostic monitoring procedure for reptilian pets – one of the final goals of the Conservation Medicine - we found that the hygienic status of a reptilian pet stock can be ascertained efficiently by performing two simple, low-budget diagnostic procedures only. Vivaria facilities on an exceeding high hygienic risk can definitely be identified – even if any evident explanation of the clustering observed is missing so far (see table “stock owner”).

4. Selected References:

(1) Hassl A. (2004): Microbiological Conservation Medicine and Exotic Pets. *Wiener Klinische Wochenschrift* 116 [Suppl 4]: 53-57.

(2) Hassl A. (2002): Gut gemeintes Fehlverhalten - falsche Obsorge für Terrarientiere. 26. Internationales Symposion für Vivaristik Litschau; *Dokumentation*: 27-30.

(3) Pedraza-Díaz S., Ortega-Mora L. M., Carrión B. A., Navarro V. Gómez-Bautista M. (2009): Molecular characterisation of Cryptosporidium isolates from pet reptiles. *Veterinary Parasitology* 160(3-4): 204-10.

(4) Deming C., Greiner E., Uhl E. W. (2008): Prevalence of cryptosporidium infection and characteristics of oocyst shedding in a breeding colony of leopard geckos (Eublepharis macularius). *Journal of Zoo & Wildlife Medicine* 39(4): 600-7.

(5) Terrell S. P., Uhl E. W., Funk R. S. (2003): Proliferative enteritis in leopard geckos (Eublepharis macularius) associated with Cryptosporidium sp. infection. *Journal of Zoo & Wildlife Medicine* 34(1): 69-75.

(6) Taylor M. A., Geach M. R., Cooley W. A. (1999): Clinical and pathological observations on natural infections of cryptosporidiosis and flagellate protozoa in leopard geckos (Eublepharis macularius). *Veterinary Record* 145(24): 695-9.

(7) Cooley W. A., Taylor M. A., Geach M. R. (1999): Demonstration of Cryptosporidia in the gut of a lizard (Eublepharis macularius) by scanning electron microscopy. *Scanning* 21(2): 141-3.

(8) Morgan U. M., Constantine C. C., Forbes D. A., Andrew Thompson R. C. (1997): Differentiation between Human and Animal Isolates of Cryptosporidium parvum using rDNA Sequencing and direct PCR Analysis. *Journal of Parasitology* 83(5): 825-83.

(9) Verweij J. J., Blange R. A., Templeton K., Schinkel J., Brienen E. A. T., vanRooyen M. A. A., van Lieshout L., Polderman A. M. (2004): Simultaneous Detection of Entamoeba histolytica, Giardia lamblia, and Cryptosporidium parvum in Fecal Samples by Using Multiplex Real-Time PCR. *Journal of Clinical Microbiology* 42(3):1220-3.