Original Article

wiener klinische wochenschrift
the middle european journal of medicine
Printed in Austria

Wien Klin Wochenschr (2003) 115 [Suppl 3]: 68–71

Hygienic evaluation of terraria inhabited by amphibians and reptiles: cryptosporidia, free-living amebas, salmonella

Andreas Hassl[1,2] and **Gerald Benyr**[3]

[1]Department of Medical Parasitology of the Clinical Institute of Hygiene and Medical Microbiology, University of Vienna, Vienna,
[2]Micro-Biology Consult Dr. Andreas Hassl, and
[3]Department of Ecology of the Natural History Museum Vienna, Vienna, Austria

Hygienische Bewertung von mit Amphibien und Reptilien besetzten Terrarien: Kryptosporidien, freilebende Amöben, Salmonellen

Zusammenfassung. Amphibien und Reptilien sind beliebte Heimtiere in etwa 90.000 österreichischen Haushalten trotz ihrer immer wieder bezweifelten Fähigkeit, einige Erkrankungen auf den Menschen zu übertragen. Wir studierten die epidemiologische Signifikanz des Dreiecks Tierhalter, exotisches Haustier und Futtermäuse mittels der Untersuchung der Häufigkeit von drei intestinalen Infestationen hervorgerufen durch Kryptosporidien, opportunistische freilebende Amöben und Salmonellen in Amphibien und Reptilien, die in einer öffentlichen Vivarienanlage leben. Neben dem Erstnachweis des Vorkommens von *Naegleria australiensis* in Österreich und des Auftretens von dieser Spezies und von *Acanthamoeba polyphaga* im Kot von Reptilien weltweit, konnten wir eine strenge Assoziation zwischen Salmonella subspecies I und in Gefangenschaft gehaltenen Reptilien und zwischen S. subspecies III und freilebenden Eidechsen finden. Der Tierhalter, die exotischen Heimtiere und die Futtermäuse könnten so ein epidemiologisches Auffangbecken für solche infektiöse Agenzien bilden, die nicht wirtsspezifisch sind. Diese neue epidemiologische Situation könnte das weitere Aufkommen von einigen opportunistischen, exotischen Erkrankungen unterstützen, wie z.B. die Reptilien-assoziierte Form der Salmonellose. Trotz der Vorteile, die eine Haltung von exotischen Heimtieren in Haushalten bringt, müssen besonders immundefiziente Personen sich der Gefahren bewusst sein, die die von den Haustieren ausgehenden Infektionskranheiten darstellen können.

Schlüsselwörter: Kryptosporidien, Amöben, Salmonellen, exotische Heimtiere, Österreich.

Summary. Amphibians and reptiles are popular pet animals in about 90.000 Austrian households despite their frequently debated capacity to transmit diseases associated with animal keeping. We studied the epidemiological significance of the triangle animal keeper, exotic pet animal, and feed mice by investigating the frequency of three intestinal infestations, caused by cryptosporidia, opportunistic free-living amebas and salmonella, in amphibians and reptiles living in a public vivarium. In addition to recording the first known occurrence of *Naegleria australiensis* in Austria, and of this species and of *Acanthamoeba polyphaga* in the feces of reptiles worldwide, we also detected a strong association between Salmonella subspecies I and captive reptiles and between S. subspecies III and free-living lizards. Thus, animal keeper, the exotic animals kept, and the feed mice may constitute an epidemiological pool for the interchange of these infectious agents. This new epidemiological situation may cause an increase of some opportunistic and exotic diseases such as reptile-borne salmonellosis. Despite the perceived benefits of keeping exotic animals in a household, the general public and especially those who have an immunodeficiency must be made aware of the danger of infectious diseases possibly being spread by their pets.

Key words: Cryptosporidia, ameba, salmonella, exotic pets, Austria.

Introduction

Pet animals offer individuals and the community significant benefits, thus, pet keeping is an ancient phenomenon, and the potential for transmission of infectious agents from these animals to humans seems to be well recognized [1]. However, the spectrum of animal species kept in central European households is changing dramatically towards exotic animals, especially reptiles, amphibians, and spiders. It has been noted that the prevalence of some pet-associated parasites, such as Giardia and Cryptosporidium, has increased over the past few decades while others, such as Toxocara and Ancylostoma, have decreased [2]. These changes can easily be explained by the increase in the numbers of exotic pets kept. Moreover, reptile-associated salmonellosis is constantly causing more attention [3]. The increasing worldwide trade in exotic pets is a serious physical threat to the animal populations and carelessly spread infectious diseases may be one of the main reasons for the worldwide decrease of amphibian populations [4].

Few, mostly preliminary, studies have been published on zoonoses originating from pet amphibians and reptiles in Austria; however, there are reports on free-living amebas [5] and salmonella [6–8]. Our special interest focuses on the hygienic condition of the interface between exotic animal, animal keeper, and animal feed, which consists mostly of mammals, especially mice. In the vivarium of the Natural History Museum Vienna, where conditions of animal keeping are more-or-less constant, we studied the epidemiology of the most important agents [Cryptosporidia, free-living (fl-) amebas and Salmonella] potentially able to interchange between the host organisms.

Material and methods

During a period of 1.5 years 350 feces samples were collected from 80 individuals of 48 species of amphibians and reptiles of various taxa (amphibians 20%; snakes 25%; lacertilia 47%, pleurodira 8%) usually kept for the purpose of exhibition in a public vivarium at the Natural History Museum Vienna. The study included only data derived from animals living in captivity for at least one month, though almost all animals were captive-bred and/or living in captivity for more than six months at the time of the survey. During the same period 56 feces samples of free-living lizards (mostly *Lacerta agilis, Zootoca [L.] vivipara, Iberolacerta [L.] horvathi, Podarcis muralis*) were collected during four excursions to nature reserve areas in Lower Austria, Carinthia and Slovenia. In addition, 24 feces samples of randomly bred feed mice (*Mus musculus*) were tested.

All fecal samples were picked up immediately after defecation, strictly avoiding any contamination with soil material. The samples were suspended 1:5 (w/v) in sterile phosphate-buffered saline pH 7.4 without any fixative, filtered through gauze to remove large particles, and microscopically screened.

A modular procedure was used for the detection of oocysts of different *Cryptosporidium* species (*C. parvum* “human”, “calf”; *C. serpentis; C. muris*) [9]. The core component was a gene amplification technique [10] in combination with microscopy; species assignment was based on amplicon-length determination. If cysts of the ameba families Vahlkampfiidae and Acanthamoebidae were detected by microscope screening, the amebas were isolated and multiplied at 24 °C, cloned, and typed according to standard techniques described by Page [12] and by genetic procedures [13]. Salmonella were isolated and identified according to the procedure described by Pfleger et al. [8] after enrichment breeding at 28 °C of 24 h in Pappaport-Vassiliadis broth (Merck KgaA, Darmstadt D).

Demographic and environmental data were analyzed to identify the source of risks. Significance values were calculated using the two-tailed Fisher’s exact test (InStat 1.15, GraphPAD, San Diego, California), matching partial cohorts (e.g. reptiles vs amphibians, fl-lizards vs. captive reptiles) or analyzing ecological and behavioral data. Only data of cohorts showing significance were processed further. Significance was defined by rounded P-values of <0.1 for marginal significance and <0.001 for extreme significance.

Results

Cryptosporidia were detected in about 5% of the feces samples of captive amphibians and reptiles, but not in feces of free-living lizards or feed mice. The species of Cryptosporidia detected were *C. parvum*, a pathogen in man and other vertebrates, in 14% of the cases, the reptile pathogenic species *C. serpentis* in 24%, *C. muris* was not found, and the species in the remaining cases of oocyst excretion (62%) was unknown.

Altogether 16 strains of fl-ameba were isolated and characterized from feces of amphibians and reptiles. Cysts of *Acanthamoeba sp.* were found in feces of *Basiliscus plumifrons* (lacertilia) and *Chelus fimbriatus* (pleurodira), cysts of *Acanthamoeba polyphaga,* multiplying at 40 °C, in the feces of *Iguana iguana* (lacertilia), cysts of a *Naegleria* species were found in feces of *Varanus prasinus, Pogona vitticeps* and *Oplurus cuvieri* (all lacertilia), and *Xenopeltis unicolor and Thamnophis radix* (snakes). A thermotolerant (multiplication at 40 °C) strain of *Naegleria australiensis* was detected in feces of an *Iguana iguana* (lacertilia*), Echinamoeba sp.* were found in a *Varanus prasinus* (lacertilia)*, Hartmanella vermiformis* and a thermotolerant strain of *Vahlkampfia lobospina* in a *Laudakia stellio* (lacertilia). Even amphibians excrete cysts of fl-amoeba: *Vahlkampfia lobospina* was found in the feces of the toad *Bufo viridis,* and a frog, *Xenopus clivii*, excreted cysts of *Naegleria gruberi.*

Various strains of Salmonella subspecies I were isolated [8]. The Salmonella subspecies III (*S. arizonae*) was characterized only to the species level. This species was exclusively found in the feces of captive snakes, *Elaphe obsoleta* and *Pituophis melanoleucus*, and in highly significant frequency in the feces of free-living lizards.

Calculation of results from cohorts formed on the basis of feed other than mammals, terraria type, dampness, host taxon, season, or other intestinal parasites did not produce usable data.

Discussion

Keeping easy-to-handle exotic animals such as some amphibians and reptiles is a very popular leisure activity in Central Europe. These exotic pet animals are kept in about 90.000 Austrian households, giving about 220.000 persons immediate access to them. However, knowledge about the potential risks of exchange of infectious agents between these pets, the human keepers, and the feed animals, especially if mammals are fed, is only moderate. Although there is a large quantity of literature dealing with Salmonella and exotic pets (referred in [14]), data on other agents causing zoonotic diseases are scarce. We have selected three agents, one obligatory parasite, an opportunistic ameba, and a bacterium thought to be closely associated with reptiles, to characterize the epidemiology of the interface between humans, captive exotic animal, and feed mammals.

Captive reptiles have been found to shed oocysts of *C. parvum*, which are infective to humans [15], and Graczyk et al. [16] suggest zoonotic transmission of cryptosporidia via water from lower vertebrates to humans. Consequently, humans may well be the source of cryptosporidia infestations of exotic pets, especially the species *C. parvum,* known to be pathogenic in humans, mice, and reptiles. The reptile parasite *C. serpentis* is regularly detected in the animal stock of a vivarium and seems to be associated with immunosuppression in reptiles, similarly to the association between *C. parvum* “human” with humans. As *C. muris* was not found, and the feed mice seem to be free of cryptosporidia, introduction of this apicomplexa via mammals fed to the reptiles seems to be unlikely. Our data indicate a low contamination rate of lizards living in nature reserve areas. Thus, we have to postulate a human origin of the Cryptosporidia infestations in the captive herpetotaxa, most likely attributable to long-lasting immunodeficiency due to the abnormal old age of specimens kept in vivaria or, though less likely, to high stress produced during handling. There is great uncertainty about the relationship between cryptosporidia and amphibians because investigators may have confused oocysts with the fresh water algae *Oocystis sp.* Nevertheless, the data presented here and previously published data [17] support the thesis of cryptosporidia excretion in frogs.

The occurrence of fl-amebas in the intestinal content of amphibians and reptiles is a topic of contradictory discussion; these amebas seem to be ubiquitous organisms, therefore ingestion and undigested shedding of cysts (pseudoparasitism) are supposed by some (cit. in [18]); in contrast, parasitism or transitory colonization are proposed by others. Based on our detection technique, we believe we detected cases of true amebic parasitism or colonization and not cases of pseudoparasitism. Furthermore we agree with Madrigal Sesma et al. [18], who postulate colonization of the intestinal system of the host animal when there are detectable numbers of ameba cysts in the feces. Nevertheless, proof of true amebic intestinal parasitism is lacking and cannot be provided, despite the incidental finding of trophozoites. Clinically striking disease is not associated with fl-ameba excretion, an observation made by us and by others (e.g. [19]). Moreover, the occurrence of fl-amebas in the feces of reptiles and amphibians kept long-term (3.7%) is a rare event compared with the incidences of 42–93% found in populations of free-living animals [20]. There are some remarkable aspects to our findings. Firstly, there seems to be no correlation between the taxon of the host and the excretion of fl-ameba cysts. This finding indicates that the anatomical and physiological differences of cold-blooded animals, which correlate with the phylogeny, do not seriously influence the ability of fl-amebas to colonize the gut. Secondly, as amebas need moisture for activity and multiplication, freshwater animals such as amphibians should be colonized more frequently than others. Nevertheless, animals living in terraria with an arid climate seem to be colonized by fl-amebas roughly as frequently as animals from other terrarium types (data not shown). This is in contrast to findings in free-living animals [18, 20]. In a study by Madrigal Sesma et al. [18] excretion of cysts was less frequent in the frog *Rana perezi* than in desert reptiles, and in the wilderness ameba cysts have easily and frequently been isolated from hot desert sands [21]. Thirdly, there is a highly significant correlation between the occurrence of fl-amebas and *Pseudomonas sp.* Pseudomonades are superb nourishment for some fl-amebas [22]. It remains unclear whether the occurrence of these bacteria is a prerequisite for intestinal colonization by fl-amebas or an expression of similar environmental requirements.

As fl-amebas are common and ubiquitous protozoa, colonization of the intestine of the captive herpetotaxa is via ingestion of water, regardless of any human interference. To our knowledge, ours is the first report on the occurrence of the ameba species *Naegleria australiensis* in Austria, of this species and *Acanthameba polyphaga* in the feces of reptiles worldwide, and of *Naegleria gruberi* in the feces of an amphibium (*Xenopus clivii*).

Salmonella is a well known, mostly harmless, companion of exotic pets (e.g. [23]). Some authors claim that Salmonella species seem to be an essentially normal component of reptilian intestinal flora, as 90% or more of reptiles harbour salmonellae [3]. But we doubt whether, undifferentiated, all subspecies of Salmonella are part of the normal intestinal flora of reptiles, as the results of European investigations on the frequency of Salmonella infestations in captive reptiles do not support this view ([6, 8], own results). Salmonella subspecies III (*S. arizonae*) is said to be associated with snakes [3]. Our data

Table 1. Detection of opportunistic parasites and salmonella in feces samples of amphibians, reptiles, and feed mice

Feces sample	Cryptosporidia				fl-Amebas			Salmonella subspecies I			Salmonella subspecies III		
	n	pos n	pos %	P	pos n	pos %	P	pos n	pos %	P	pos n	pos %	P
Captive herpetotaxa	350	17	4,9		13	3,7		49	14		4	1,1	
> Amphibians	53	2	3,8	1,000	2	3,8	0,999	3	5,7	**0,082**	0	0	0,627
> Reptiles	297	15	5,1		11	3,7		46	15,5		4	1,3	
Herpetotaxa feeding on mice	168	nd			5	1,5	**0,05**	34	20,2	**0,002**	4	2,4	0,999
Herpetotaxa colonized with pseudomonas	9/75	nd			7		**0.00014**	nd			nd		
Free-living lizards	56	0	0	0,142	3	5,4	0,706	2	3,6	**0,017**	17	30,4	**< 0,00001**
Feed mice	24	0	0		0	0		0	0		0	0	

Significance values were calculated by comparing matching cohorts, but only clusters containing significance (bold) and extreme significance (underlined) are shown; *nd* no data.

indicate that Salmonella subspecies III is found in free-living reptiles and lizards, with extremely significant probability, whereas subspecies I seems to be associated with the keeping of reptiles and amphibians by humans, perhaps covering up infestations with other species or subspecies. Thus, infestations of captive reptiles and amphibians with Salmonella subspecies I may be caused far more often by human interference than by native circumstance. This thesis is also supported by the weak association between Salmonella infestation and feeding animals with mice, although no Salmonella was ever isolated from feces of feed mice, and by the marginally significant absence of Salmonella subtype I in free-living lizards. No simple explanation was found for the rate of Salmonella infestation of reptiles being three times higher than in amphibians. In summary, Salmonella subspecies III may be common in the intestine of free-living reptiles but they are rapidly and almost completely replaced by Salmonella subspecies I when humans interfere.

Cryptosporidia infestations in pet animals seem to be a good example of the epidemiological changes caused by humans keeping such animals long-term and the consequent germ exchange. The diseases caused by reptile-borne salmonellosis are attributable to unsatisfactory conditions of hygiene during keeping and unsatisfactory human behavior. The keeper, the exotic pets, and the feed animals, especially if the latter are bred by the keeper, may form an epidemiological reservoir for the exchange of agents. However, the controversial role of humans in the phenomenon of declining amphibian populations may not be associated with changes in the epidemiology of infectious diseases by keeping amphibians as pets. Nevertheless, feces of captive exotic pet animals must be considered as a health hazard to man, especially to immunosuppressed persons. Thus, keeping exotic pets cannot be recommended unrestrictedly despite the perceived benefits.

Acknowledgements

Mag. Silvia Pfleger and Dr. Julia Walochnik kindly cooperated in data acquisition, some members of the Austrian Herpetological Society and the Austrian Section of the Declining Amphibian Populations Task Force supported us with ideas, and Mrs. Susanne Rudnicki, Mrs. Ingrid Blöschl and Mrs. Ilse Veits gave excellent technical assistance. The authors cordially thank all supporters.

References

1. Frank W (1986) Hygienic problems and pet animals in the Ferderal Republic of Germany. Zbl Bakt Hyg B 183: 274–303
2. Robertson ID, Irwin PJ, Lymbery AJ, Thompson RCA (2000) The role of companion animals in the emergence of parasitic zoonoses. Int J Parasitol 30: 1369–1377
3. Warwick C, Lambiris AJL, Westwood D Steedman C (2001) Reptile-related salmonellosis. J Royal Soc Medicine 94: 124–126
4. Carey C, Cohen N, Rollins-Smith L (1999) Amphibian declines: an immunological perspective. Develop Comp Immunology 23: 459–472
5. Hassl A, Benyr G, Appelt S (2000) Freilebende Amöben als opportunistische Darmparasiten von Reptilien. Mitt Österr Ges Tropenmed Parasitol 22: 49–54
6. Geue L, Loschner U (2002) Salmonella enterica in reptiles of German and Austrian origin. Vet Microbiol 84: 79–91
7. Gumpenberger M (2000) Reptilien und Salmonellen aus veterinärmedizinischer Sicht. Mitt Österr Ges Tropenmed Parasitol 22: 55–58
8. Pfleger S, Benyr G, Sommer R, Hassl A (2003) Pattern of salmonella excretion in amphibians and reptiles in a vivarium. Int J Hyg Environ Health 206: 53–59
9. Hassl A, Vorbeck-Meister I, Sommer R, Rotter M (1999) Nachweis und Identifikation von Kryptosporidien in Kot-, Stuhl- und Umweltproben: Entwicklung einer Modultechnik. Mitt. Österr Ges Tropenmed Parasitol 21: 45–50
10. Hassl A, Benyr G, Sommer R (2001) Occurrence of Cryptosporidium sp. oocysts in feces and water samples in Austria. Acta Tropica 80: 145–149
11. Morgan UM, Constantine CC, Forbes DA, Thompson RC (1997) Differentiation between human and animal isolates of Cryptosporidium parvum using rDNA sequencing and direct PCR analysis. J Parasitol 83: 825–830
12. Page FC (1991) Nackte Rhizopoda. In: Matthes D (Hrsg) Protozoenfauna, Band 2. G Fischer, Stuttgart New York, pp 7–170
13. Walochnik J, Hassl A, Simon K, Benyr G, Aspöck H (1999) Isolation and identification by partial sequencing of the 18S ribosomal gene of free-living amoebae from necrotic tissue of Basiliscus plumifrons (Sauria: Iguanidae). Parasitol Res 85: 601–603
14. Woodward DL, Khakhria R, Johnson WM (1997) Human salmonellosis associated with exotic pets. J Clin Microbiol 35: 2786–2790
15. Arcay L, DeBorges EB, Bruzual E (1995) Cryptosporidiosis experimental en la escala de vertebrados. I. Infecciones experimentales. II. Estudio histopathologico. Parasitol al Dia 19: 20–29
16. Graczyk TK, Fayer R, Cranfield MR (1997) Zoonotic transmission of Cryptosporidium parvum. Implications for water-borne cryptosporidiosis. Parasitol Today 13: 348–351
17. Hassl A (1991) An asymptomatic cryptosporidia (apicomplexa: coccidia) infection in Agalychnis callidryas (COPE, 1862) (Anura: Hylidae). Herpetozoa 4: 127–131
18. Madrigal Sesma MJ, Chamarro Garcia L, Guillen LP (1988) Free-living amoebas and cold-blooded animals. J Parasitol 74: 883–884
19. Frank W, Bosch I (1972) Isolierung von Amöben des Typs „Hartmanella-Acanthamoeba“ und „Naegleria“ aus Kaltblütern. Z Parasitenk 40: 139–150
20. Madrigal Sesma MJ, Ramos LZ (1989) Isolation of free-living amoebas from the intestinal contents of reptiles. J Parasitol 75: 322–324
21. Lastovica AJ (1980) Isolation, distribution, and disease potential of Naegleria and Acanthamoeba (order: Amoebida) in South Africa. Trans Royal Soc South Africa 44: 269–278
22. Hall J, Voelz H (1985) Bacterial endosymbionts of Acanthamoeba sp. J Parasitol 71: 89–95
23. Chiodini RJ, Sundberg JP (1981) Salmonellosis in reptiles: a review. Am J Epidem 113: 494–499

Correspondence: Andreas Hassl, PhD, Department of Medical Parasitology, Clinical Institute of Hygiene and Medical Microbiology, University of Vienna, Kinderspitalgasse 15, A-1095 Vienna, Austria,
E-mail: andreas.hassl@univie.ac.at