Available online at www.sciencedirect.com
ScienceDirect
The Veterinary Journal 175 (2008) 53–68
The Veterinary Journal
www.elsevier.com/locate/tvjl

Review

Introducing reptiles into a captive collection: The role of the veterinarian

Frank Pasmans [a,*], Silvia Blahak [b], An Martel [a], Nikola Pantchev [c]

[a] Department of Pathology, Bacteriology and Avian Diseases, Ghent University, B-9820 Merelbeke, Belgium
[b] Veterinäruntersuchungsamt OWL, Westerfeldstrasse 1, 32758 Detmold, Germany
[c] Veterinary Laboratory Ludwigsburg, Division of IDEXX Laboratories, Mörikestrasse 28/3, D-71636 Ludwigsburg, Germany

Accepted 14 December 2006

Abstract

The successful introduction of reptiles into a captive collection depends on providing optimal husbandry and veterinary attention. An important role of the veterinarian in this process is the prevention of disease introduction, which may affect both the introduced and the resident animals. This review focuses on preventive veterinary medicine in reptiles, emphasising quarantine measures, disinfection and entry control for infectious agents. Agents discussed include those that are likely give rise to severe clinical problems on introduction into a collection of reptiles, or, in the case of *Salmonella*, those that pose a significant public health risk. Aetiology, clinical signs and diagnosis are discussed for the most relevant endo- and ectoparasites, bacteria and viruses including *Cryptosporidium* and *Entamoeba*, *Salmonella*, *Dermabacter*, Chlamydiales, *Mycoplasma*, *Herpesvirus*, *Adenovirus*, *Paramyxovirus* and inclusion body disease.

Keywords: Reptile; Infectious diseases; Prevention; Entry control

1. Introduction

Reptilian medicine has evolved greatly in recent decades. However, the acquisition of reptiles, either as captive bred or wild caught animals, yields a high risk of disease introduction into a collection. Routine screening and subsequent treatment by a skilled veterinarian of every recently acquired reptile could greatly contribute to the survival rate of these animals in captivity.

Management and clinical examination techniques are of critical importance but these topics are covered in detail elsewhere. In this article, we will focus on preventive veterinary medicine in reptiles, emphasising quarantine measures as well as disinfection and entry control for infectious agents. Agents will be discussed that are most likely to give rise to severe clinical problems upon introduction into a collection of reptiles, or, in the case of *Salmonella*, that pose a significant public health risk.

2. Quarantine

The importance of quarantine can hardly be exaggerated, yet most reptile and amphibian keepers do not respect any quarantine period and even where it is imposed adequate measures are rarely taken. Infectious agents do not respect terrarium boundaries and newly acquired animals should be housed separately from a resident collection. With some viral infections, such as paramyxovirus, it is not sufficient just to place a new animal in a separate terrarium because, as the virus is transmitted by air, the quarantine terrarium must be in a separate room.

Newly acquired reptiles should always be treated after the resident collection has been dealt with. Disposable gloves should be worn and hands must be disinfected after every contact with the animals or their surroundings. Instruments (dip nets, forceps, snake hooks, etc.) should

* Corresponding author. Tel.: +32 92647441; fax: +32 92647494.
E-mail address: frank.pasmans@UGent.be (F. Pasmans).

1090-0233/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tvjl.2006.12.009

be kept strictly separate from those used for the husbandry of the resident collection and must be thoroughly cleaned and disinfected after every use.

Ideally, new inmates should be housed in isolation, enabling individual health checks to be performed and eliminating the possibility of intraspecies aggression. The quarantine container should be designed to be easy both to inspect and to clean thoroughly, yet respecting the environmental needs of the species concerned (heat, light, humidity, shelter, etc.). The bedding must be easy to change: journal paper meets these requirements and allows easy evaluation of food intake and faecal consistency. Live plants are generally not suitable for quarantine containers.

Importantly, wild caught animals should be handled as little as possible to facilitate acclimation. Acclimating wild caught reptiles to become long term captives remains a challenge and such animals benefit greatly from an environment providing ample opportunity for them to feel secure.

Generally, quarantine should last for at least 90 days (Jacobson et al., 2001), but there are some exceptions. Boid snakes, for example, require a quarantine period of 6 months because of the high prevalence of inclusion body disease (IBD) in snake collections. Tortoises should be kept separate both during hibernation and for the first 4–6 weeks after awakening, as it is during this period that most outbreaks of herpesvirus infections occur reflecting a lowering of the immune response during hibernation.

To find out whether a newly acquired reptile is a silent carrier of viral infection, investigations for the presence of antibodies are recommended. In terrestrial tortoises, blood samples can be checked for antibodies against herpesvirus, preferably on two occasions during the quarantine period. Colubrids, elapids and viperids should be monitored for antibodies against ophidian paramyxovirus. The presence of antibodies indicates that the reptiles survived an infection but they may however be carriers of latent virus. Boid snakes of the subfamily Boinae can be investigated with a blood smear or a liver biopsy for intracytoplasmic inclusions, indicating IBD.

In addition to a thorough veterinary check, repeated faecal examinations (at the beginning, during and the end of the quarantine period) should be performed to monitor intestinal parasitic load. Parasitic infections that are actually easy to diagnose and treat still account for high mortality rates in reptiles, even in captive breds, which are frequently exposed to suboptimal conditions, for example in pet shops. Every endeavour should be made to try to convince the owner to necropsy every dead animal and arrange for bacteriological and virological investigations to be undertaken.

Quarantine measures should be applied particularly vigorously where an animal has been obtained from a wholesale trader or pet shop, which both provide ideal conditions for infectious agents to spread freely. High stocking densities, continuous flow of animals, often very highly stressed and diseased animals offer a plethora of opportunities for cross-contamination through staff and equipment.

3. Disinfection

An overview of disinfectants for use in reptile and amphibian enclosures has been provided by Slomka-McFarland (2006). It is important to remember that the less organic debris and the longer the contact time with the disinfectant, the more efficient it will be. The present authors have had good experience with 4-chlor-M-cresol and organic acids (Neopredisan; Eckert et al., 2005) and a 5% dilution of sodium hypochlorite (household bleach). *Cryptosporidium* contaminated objects can be also treated with 5% ammonium solution (effective in eliminating oocyst infectivity after 18 h of contact at 4 °C; Cranfield et al., 1999) and should be allowed to dry for at least 3 days after that. This solution does not directly kill oocysts but leads to their encystation. After 3 days, unprotected free sporozoits will dry up.

Disinfectants based on phenol or quaternary ammonium are not appropriate for use in the reptile environment (Köhler, 1996) and any treated surfaces should be thoroughly rinsed and aired after the recommended incubation time to prevent any direct contact of a reptile with these disinfectants.

Chemical disinfectants should be applied only to empty cages and following the manufacturers' instructions. Cryptosporidia oocysts (Fig. 1) can be inactivated by heat (45–60 °C for 5–9 min; Cranfield et al., 1999) and temperatures of >52 °C will inactivate cysts of *Entamoeba invadens* (Bonner et al., 2001).

Disinfection of terrarium and furnishings for most ectoparasites can be carried out using fipronil (Frontline Spray, Merial). Surfaces should be allowed to dry thoroughly. Snake mites do not survive temperatures >45 °C and <5 °C or humidity <20% (Camin, 1953). It is recommended

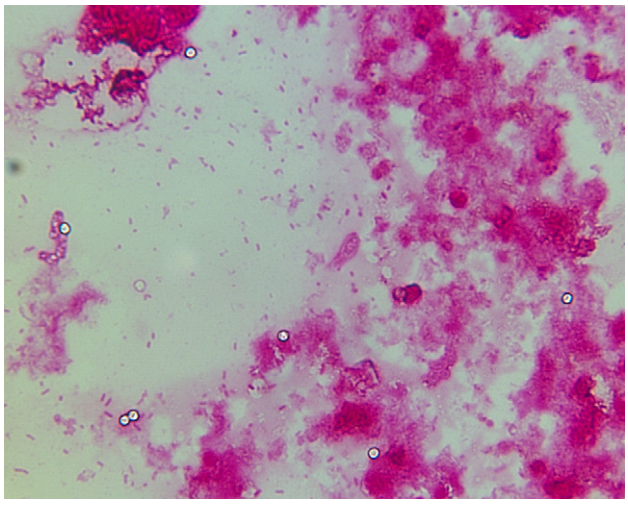

Fig. 1. Oocysts of *Cryptosporidium* spp. (5.0 × 4.5 μm) stained with carbol-fuchsin.

that the whole room should be cleaned thoroughly using a vacuum in order to remove any migrating mites. In snakes and lizards, ivermectin can replace fipronil and can be used to decontaminate the terrarium. Ivermectin, available as a 1% solution, should be diluted by adding 1 mL (10 mg) to 1 L of water; this solution is stable for up to 30 days (Kahn, 2005).

4. Entry control for endoparasites in reptiles

Endoparasites are an important cause of disease in captive reptiles. These include Apicomplexa (mainly *Isospora* (Fig. 2) and *Eimeria*), flagellate protozoa (e.g. *Hexamita*, trichomonads), nematodes (Fig. 2), cestodes (Fig. 3) and trematodes. Cryptosporidiosis and amoebiasis are considered separately. Many intestinal parasites, such as the ciliate *Balantidium*, may be normal residents of the intestinal microbiota but can also be involved in gastrointestinal disease.

Endoparasite infections cause very different clinical symptoms depending on the parasite species and the degree of infestation. In severe cases, developmental disorders or even mortality may occur. In 4000 reptile post-mortem examinations, parasite infection was detected in 50% of the animals compared to 15% in bird and 32% in mammal necropsies (Ippen, 1992). A retrospective study at the Detroit Zoo from 1973–1983 showed that 12% of 1300 reptiles that died during this period were lost due to parasite infections caused by, for example, lungworms or *Entamoeba* spp. (Kaneene et al., 1985).

At the Ludwigsburg Veterinary Laboratory, samples of 2267 reptiles were examined during 2003–2005 using routine faecal examination. The results are summarised in Tables 1 and 2. These tables do not consider the prevalence of all spurious parasites in reptiles, e.g. rodent specific parasites that are particularly common in faeces of snakes

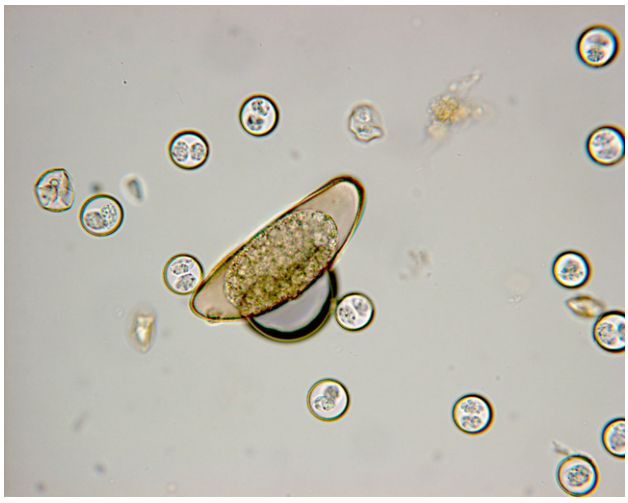

Fig. 2. Oxyurid egg (centre) and oocysts of *Isospora* spp. from *Chlamydosaurus kingii*, 400×.

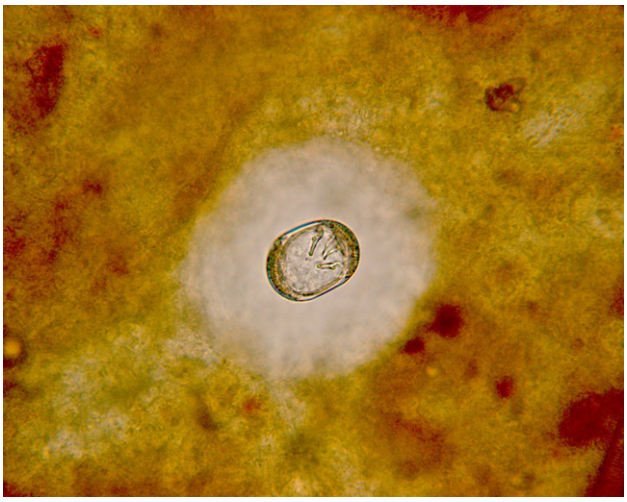

Fig. 3. Cestode egg (*Oochoristica* spp.) from *Iguana iguana*, 400×.

Table 1
Prevalence of endoparasites in captive chelonians

$n = 1251$ (Testudinidae:Emydidae:unspecified Chelonia = 24%:3%:73%)		
Chelonians		
Oxyurids	48.2%	$n = 603$
Ascarids (*Angusticaecum holopterum*)	2.7%	$n = 34$
Heterakids	0.2%	$n = 2$
Capillarids[a]	0.5%	$n = 6$
Pentastomids	0.1%	$n = 1$
Flagellates (Trichomonadida)	18.6%	$n = 233$
Flagellates (*Hexamita* spp.)	3.4%	$n = 43$
Ciliates (*Nyctotherus* spp.)	11.5%	$n = 144$
Ciliates (*Balantidium* spp.)	12.8%	$n = 160$
Amoebas (*Entamoeba* spp.)	4.3%	$n = 53$
Amoebas (*Entamoeba invadens*)	2.6%	$n = 33$
Coccidia (*Isospora* spp.)	0.2%	$n = 2$

[a] Probably bird specific spurious parasites.

(Pantchev, 2005). During 2003–2006, *Cryptosporidium* spp. were identified in 44/243 (18%) examined samples using a carbol-fuchsin staining technique (Fig. 1) and the ProSpecT *Cryptosporidium* Microplate Assay (Coproantigen ELISA). Twenty-five (56.8%) of the positive samples were from lizards (18 leopard geckos *Eublepharis maculatus*, three collared lizards *Crotaphytus* spp., two chameleons, one bearded dragon *Pogona vitticeps* and an unspecified lizard); there were 17 (38.6%) positives from snakes (seven corn snakes *Elaphe guttata*, one *Boa constrictor* and nine unspecified snakes) and there were two positives (4.6%) from chelonians (a black-breasted leaf turtle *Geoemyda spengleri* and a Russian tortoise *Testudo horsfieldii*).

The high prevalence of nematodes such as oxyurids, *Rhabdias*/*Strongyloides* spp. and *Kalicephalus* spp., or protozoa such as *Isospora* spp., *Eimeria* spp. and *Cryptosporidium* spp. can be explained by their direct life cycle (with no intermediate host) and the high tenacity of eggs, larvae or oocysts to survive in the environment, which

Table 2
Prevalence of endoparasites in captive lizards and snakes

Lizards	Oxyurids	Ascarids/heterakids	*Rhabdias/Strongyloides*	Spirurids	Cestodes	Trematodes	Pentastomids	Trichomonadida	*Isospora*	(Choleo)-*Eimeria*	*Nyctotherus*	*Balantidium*	Amoebas
Agamidae[a]	61.6%	1.5%	1.3%	0.5%	0.3%	0.3%	–	11.6%	43.5%	1.5%	13.9%	–	1.0%[b]
$n = 386$	($n = 238$)	($n = 6$)	($n = 5$)	($n = 2$)	($n = 1$)	($n = 1$)		($n = 45$)	($n = 168$)	($n = 6$)	($n = 54$)		($n = 4$)
Iguanidae[c]	43.2%	0.6%	–	1.2%	11.6%[d]	–	–	20.2%	0.6%	1.8%	3.0%	5.5%	20.7%[e]
$n = 164$	($n = 71$)	($n = 1$)		($n = 2$)	($n = 19$)			($n = 33$)	($n = 1$)	($n = 3$)	($n = 5$)	($n = 9$)	($n = 34$)
Chamaeleonidae	27.3%	6.0%	1.5%	1.5%	–	4.5%	–	13.6%	7.6%	3.0%	1.5%	–	–
$n = 66$	($n = 18$)	($n = 4$)	($n = 1$)	($n = 1$)		($n = 3$)		($n = 9$)	($n = 5$)	($n = 2$)	($n = 1$)		
Gekkonidae	51.5%	2.9%	2.9%	1.5%	2.9%	–	5.8%[f]	14.7%	–	10.3%[g]	14.7%	1.5%	–
$n = 68$	($n = 35$)	($n = 2$)	($n = 2$)	($n = 1$)	($n = 2$)		($n = 4$)	($n = 10$)		($n = 7$)	($n = 10$)	($n = 1$)	
Varanidae	4.7%	–	14.3%	–	19.0%[h]	–	4.7%	28.6%	–	4.7%	–	–	–
$n = 21$	($n = 1$)		($n = 3$)		($n = 4$)		($n = 1$)	($n = 6$)		($n = 1$)			

Snakes	*Kalicephalus*	Ascarids/heterakids	*Rhabdias/Strongyloides*	Capillarids	Spirurids	Oxyurids	Cestodes	Trematodes	Coccidia	Trichomonadida	*Entamoeba* -spp.
Boidae[i]	8.6%	6.4%	10.0%	7.8%	1.4%	2.8%	4.3%	1.4%	3.6%[j]	13.6%	5.0%
$n = 140$	($n = 12$)	($n = 9$)	($n = 14$)	($n = 11$)	($n = 2$)	($n = 4$)	($n = 6$)	($n = 2$)	($n = 5$)	($n = 19$)	($n = 7$)
Colubridae	3.2%	1.1%	3.2%	–	–	1.1%	–	–	–	7.4%	2.1%
$n = 95$	($n = 3$)	($n = 1$)	($n = 3$)			($n = 1$)				($n = 7$)	($n = 2$)
Unspecified snakes[k]	2.6%	2.6%	–	–	–	–	–	–	1.3%	14.5%	2.6%
$n = 76$	($n = 2$)	($n = 2$)							($n = 1$)	($n = 11$)	($n = 2$)

[a] Additionally, 0.3% ($n = 1$) with *Hexamita* spp. and 0.3% ($n = 1$) with *Capillaria* spp.
[b] *Entamoeba* spp.
[c] Additionally, 0.6% ($n = 1$) with *Hexamita* spp.
[d] *Oochoristica* spp.
[e] Mainly *Naegleria* spp.
[f] *Raillietiella* spp.
[g] *Eimeria tokayae*.
[h] *Duthiersia* spp. and *Acanthotaenia* spp.
[i] Additionally, 0.7% ($n = 1$) with pentastomids.
[j] *Sarcocystis* spp., *Choleoeimeria* spp. and *Caryospora* spp.

leads to efficient transmission particularly among animals in captivity. Although the pathogenicity of the different parasites varies, unfavourable husbandry conditions (e.g. crowding or insufficient hygiene management) can lead to clinically relevant mass infestation in a terrarium due to frequent reinfection. Stressful conditions in captivity can exacerbate existing parasite infections (Grego et al., 2004).

An examination for endoparasites should be performed as part of the routine check of all reptiles. It is of vital importance that samples for examination are as fresh as possible (preferably <3 h) and fresh, moist faeces should be placed in a vial or Ziplock bag, and sent to the diagnostic laboratory directly after collection. Faeces that have dried on the bedding are not suitable for examination because parasitic stages may have degenerated, making a correct diagnosis difficult or even impossible. Preferably, several consecutive faecal samples should be checked during quarantine including specific examinations for amoebas and *Cryptosporidium* spp. Only parasite-free or successfully treated animals (two negative samples at a 2-week interval must be achieved) should be introduced into a healthy collection.

Routine faecal examination in reptiles should include flotation (zinc chloride/sodium chloride solution with specific gravity of 1.3), direct saline smear with a small volume of faeces and staining of fresh sample with iodine solution (reviewed by Pantchev, 2005). Egg- and oocyst-counting techniques (to measure EPG or OPG) may be applied in principle to any parasitic infection of any host, but they do not allow conclusions to be drawn about the burden of parasite infection in the gut. For that reason, these techniques find their greatest utility in veterinary medicine mainly in estimating the levels of strongyle infections in ruminants and horses (Bowman, 2003), for which the epidemiology is well defined. In reptiles, and especially in lizards and snakes, routine parasitic examination of the oral cavity using a saline smear from a mouth swab, is strongly advised and often reveals infections (e.g. with trichomonads) that would otherwise go unnoticed. These may play an (underestimated) role in the development of stomatitis. The examination is also a simple way to identify nematode larvae (e.g. lung worms like *Rhabdias* spp.), while avoiding more stressful (but reliable) techniques such as tracheal washes.

4.1. Cryptosporidium spp.

Unlike other animals in which infection with *Cryptosporidium* spp. is usually self limiting in immunocompetent individuals, cryptosporidiosis in reptiles is frequently chronic and sometimes lethal. The predominant sign in snakes is gastric hyperplasia with persistent

or periodical postprandial regurgitation of food animals and, in lizards, chelonians or snakes, a chronic debilitating enteritis without gastric involvement (Cranfield et al., 1999). Nine *Cryptosporidium* species have been reported in reptiles (Xiao et al., 2004) with the two most frequently found species being *C. serpentis* (mainly associated with snakes) and *C. saurophilum* (mainly associated with lizards).

Caution is advisable if the intention is to introduce into a *Cryptosporidium*-negative collection reptiles that previously tested positive for *Cryptosporidium* spp. Drug treatment frequently leads only to a clinical improvement or only temporarily terminates the shedding of oocysts into the environment, but does not completely eliminate the infection (Pantchev, 2005) and renewed shedding may occur later on. Cranfield and Graczyk (1994) reported that *C. serpentis*-negative snakes kept in separate cages but in the same room became *C. serpentis*-positive from an undetermined pathogen source within 24–45 weeks suggesting that prevention of pathogen transmission within an ophidian collection is difficult and re-emphasising the point that positive snakes must not be introduced to negative collections.

Cryptosporidium spp. can be demonstrated using the ProSpecT *Cryptosporidium* Microplate Assay (Coproantigen ELISA), immunofluorescence antibody test (IFA; e.g. Meriflour) or using an oocyst staining technique with or without concentration of the oocysts e.g. the fast carbol-fuchsin stained faecal smear test (Heine, 1982) (Fig. 1). Immunoassays utilising monoclonal antibodies developed for the detection of *Cryptosporidium parvum* should always be performed in combination with direct oocyst detection methods for several reasons. Lizard-derived oocysts produced consistently a very weak reaction with the Merifluor immunofluorescent antibody test (Graczyk et al., 1999). The ProSpecT *Cryptosporidium* Microplate Assay on the other hand identifies a *Cryptosporidium* specific antigen (CSA) that is produced by *Cryptosporidium* organisms as they multiply within the host intestinal tract and may not be always shed simultaneously with oocysts in reptile faeces (own observations). If cryptosporidian oocysts are found in reptile faeces, it is important to note that cryptosporidian oocysts from infected food animals can be shed in reptile faeces after feeding (e.g. up to 14 days in lizards after experimental infection with oocysts; Graczyk et al., 1996).

For snakes, a gastric lavage up to 3 days after feeding provides the best sample to determine the presence of a *Cryptosporidium* infection. Because of the intermittent shedding of oocysts in faeces, repeated faecal examinations are necessary to identify infection. Moreover, in the subclinical stage of infection (which can last for years), the numbers of oocysts in faecal samples are lower. The concentration of oocysts in faecal sample increases with volume of faeces excreted (Graczyk and Cranfield, 1996).

4.2. Entamoeba invadens

E. invadens is the causative agent of amoebic dysentery in reptiles. *E. invadens* commonly occurs in chelonians (Fig. 4) and crocodilians but rarely causes disease in these species. With few exceptions, chelonians are non-clinical carriers shedding cysts in their faeces. These cysts can survive in the environment for 14 or more days at a temperature of 8 °C, and for several days at 37 °C (McConnachie, 1955). In snakes and lizards on the other hand, *E. invadens* is highly pathogenic following ingestion of cysts from affected faeces (reviewed by Barnard and Upton, 1994).

Since treatment can be unsatisfactory by the time the disease is diagnosed, and the clinical symptoms (anorexia, weight loss, vomiting, blood/mucus in faeces, intussusception and palpable hardening of large intestine) are restricted to the terminal phase of infection, emphasis should be on prevention. In particular:

1. lizards or snakes must not be mixed with chelonians or crocodilians;
2. mixed exhibits must be avoided;
3. animals should be housed by geographic regions (e.g. boas and pythons should not be mixed);
4. invertebrate mechanical vectors such as flies or cockroaches must be controlled;
5. tools and equipment must be disinfected between cages.

E. invadens infection can be diagnosed using a saline faecal smear (particularly in any part of fresh faeces containing blood or mucus) and direct microscopic examination to observe the slowly amoeboid movements of the living trophozoites. More sensitive is the examination of stained smears (e.g. with iodine or trichrome) (Pantchev, 2005). Trophozoites of *E. invadens* possess a large single ringlike nucleus with a small central karyosome. Cysts are most likely to be found in formed faecal samples and contain

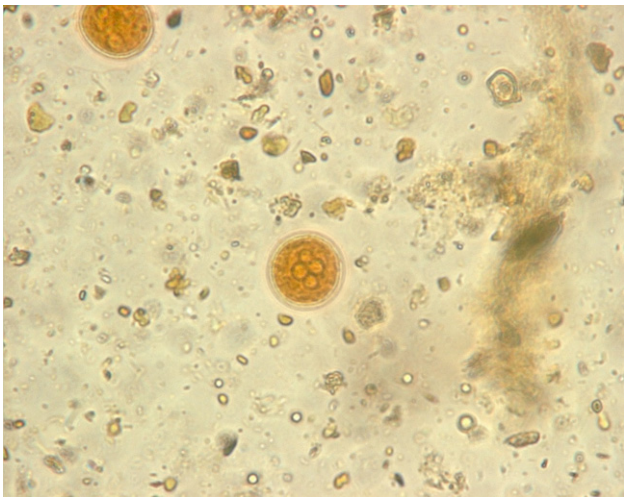

Fig. 4. Cysts of *Entamoeba invadens* (four nuclei) from a tortoise. Stained with iodine solution, 1000×.

in their mature state four nuclei (as *Entamoeba histolytica* in humans) (Fig. 4), which resemble those of trophozoites but are smaller. Such cysts should be differentiated from those of *Entamoeba* spp. with eight nuclei, which can be also observed in reptile faeces. *Entamoeba* cysts with eight nuclei (such as *Entamoeba coli* in humans or *E. muris* in rodents) are believed to be non-pathogenic and non-invasive.

Free-living amoebas as *Naegleria* or *Acanthamoeba* spp. are also found frequently in reptile faeces (see Table 2) and are thought to be spurious parasites (only passing the gut after oral uptake) or commensals which rarely cause disease (Hassl et al., 2000). The cysts of these amoebas are small and contain only one nucleus with a large karyosome. More sensitive concentration techniques for the detection of amoebas in faecal samples are the SAF (sodium acetate–acetic acid–formalin) assay (Eckert et al., 2005) and culturing in special media (Frank, 1985) with subsequent differentiation. If no faecal sample is available, saline enemas can be given followed by microscopic examination after centrifugation of the faecal fluid.

5. Entry control for ectoparasites in reptiles

Ectoparasites (ticks [Fig. 6], mites, and leeches) are very common in wild caught reptiles but also in captive breds, as many reptile collections are infected with mites. The purely blood-sucking mite *Ophionyssus natricis* is the most common reptile ectoparasite in captivity. It has a world-wide distribution affecting mainly snakes and to a lesser extent lizards, but it can also attack humans, causing papular vesiculo-bullous eruption of the skin (Schultz, 1975). The weight of an adult female may increase 15 times during a single blood meal and a heavy infestation of several hundred mites may therefore cause irritation, anorexia, skin lesions, anaemia and even death in small reptiles. *O. natricis* can move under optimal environment conditions (temperature 24–31 °C and relative humidity 70–90%) with a speed of 1.5–1.8 m/h (Camin, 1953). Therefore, gravid females are able to infect every terrarium in one room within a day.

The life cycle consists of five developmental stages (egg, larva, protonymph, deutonymph and adult) and can be completed under favourable conditions within 6 days. Unfed females are about 600 μm in length, yellowish-brown in colour, and when fully engorged may be 1300 μm in length and dark red or black. Apart from their direct pathogenicity these arthropods are able to transmit bacteria such as *Aeromonas hydrophila* among reptiles (Frank, 1985) and could transmit IBD in boid snakes. Live food animals (e.g. rodents) have been incriminated in introducing *O. natricis* into collections and refused food rodents e.g. from quarantine containers should not be offered to other reptiles in the collection. Some exotic ectoparasitic arthropod species may develop breeding colonies and become established as indigenous species and arthropod-born diseases may be spread among susceptible native populations.

Kenny et al. (2004) collected 39 ticks from 10 imported reptiles and in all ticks of the genus *Aponomma* (Fig. 5) they detected apicomplexan protozoa (*Hepatozoon* spp., Fig. 6) and in three *Aponomma* ticks they found *Ehrlichia*-like-organisms. The tick *Hyalomma aegyptium* transmits the haemogregarine *Haemolivia mauritanica* (Siroky et al., 2004) and harbours the spirochaete *Borrelia turcica* (Guner et al., 2004). The rickettsia *Ehrlichia* (*Cowdria*) *ruminantum*, the causative agent of heartwater disease in domestic and wild ruminants, has been identified in an *Amblyomma sparsum* tick on leopard tortoises imported into Florida from Zambia (Burridge et al., 2000). The soft tick *Ornithodorus talaje* can harbour the filarial *Macdonaldius oschei* and mites of the genus *Ophionyssus* are vectors for the haemogregarine protozoa *Karyolysus* (Frank, 1985).

Ectoparasites can be detected by thorough examination (preferably with magnification) of the skin with special attention given to the axillary, ear and groin regions. The

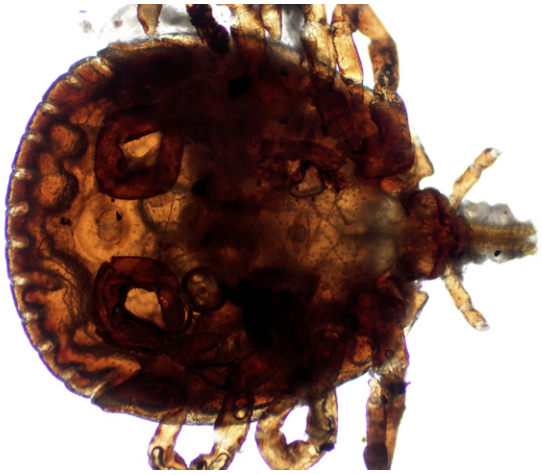

Fig. 5. Male hard tick (*Aponomma latum*) from *Python regius*, 40×.

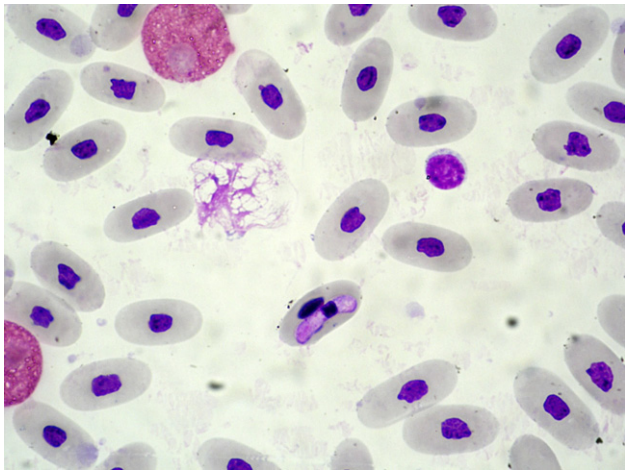

Fig. 6. Intraerythrocytic gametocyte of *Hepatozoon* spp. (blood smear) of wildcaught *Corallus hortulanus*, 1000×.

use of adhesive tape to sample the skin aids in detecting mild infections.

6. Entry control for bacteria in reptiles

Very few bacteria have been implicated in reptile diseases as primary causative agents. Most clinical bacterial infections tend to be secondary, for example to viral infections or as a result of poor husbandry. Routine swabbing of the mouth and/or cloaca to assess microbial growth and to identify reptilian pathogens is not generally recommended. Bacteria (e.g. *Salmonella*) and yeasts (e.g. *Candida*), considered pathogenic for homeothermic animals, are highly prevalent in mouth and cloacal swabs taken from reptiles (Kostka et al., 1997). Swabbing the oral cavity of clinically healthy snakes can yield all kinds of results from abundant bacterial growth including *Pseudomonas, Salmonella, Enterobacter* to a total absence of bacteria (Göbel, 1990; F. Pasmans and A. Decostere, unpublished results). It is therefore extremely difficult to interpret bacterial growth of mouth and/or cloacal swabs correctly. On the other hand, routine screening of the mouth for the presence of parasites is far more important.

6.1. Chlamydiales

Chlamydiales, notably *Chlamydophila psittaci*, have been increasingly recognized as causative agents of disease in reptiles. The order Chlamydiales consists of four families and the family Chlamydiaceae is divided into two genera, *Chlamydia* (*C.*) and *Chlamydophila* (*Cp.*) and nine species (*C. trachomatis*, *C. suis*, and *C. muridarum* and *Cp. psittaci*, *Cp. pneumoniae*, *Cp. felis*, *Cp. pecorum*, *Cp. abortus*, and *Cp. caviae*) (Everett et al., 1999). Chlamydiae are obligate intracellular pathogens and although very little is known concerning the pathogenicity of Chlamydiales for reptiles and the pathogenesis in these animals, these organisms may account for more cases than previously supposed.

Chlamydiales infections have been reported in various reptiles including Emerald tree boas (*Corallus caninus*), puff adders (*Bitis* spp.), *Elaphe taeniura*, green sea turtles (*Chelonia mydas*), chameleons (*Chamaeleo dilepis*), iguanas (*Iguana iguana*) and Nile crocodiles (*Crocodylus niloticus*) (Jacobson et al., 1989, 2004; Jacobson and Telford, 1990; Bodetti et al., 2002; Zwart and Vanrompay, 2004; F. Pasmans, unpublished data). The zoonotic serovar *Cp. psittaci* serovar A has been detected during an outbreak of disease in tortoises (Vanrompay et al., 1994) and Bodetti et al. (2002) detected several chlamydial species including *Cp. pneumoniae*, *Cp. abortus*, *Cp. felis* and *Neochlamydia* in reptile tissues. Soldati et al. (2004) demonstrated the presence of Chlamydiales in 64.4% of reptiles with granulomatous lesions. Ten percent of these positive cases showed similarity to *Cp. pneumoniae* and 54.4% to *Parachlamydia acanthamoeba* and *Simkania negevensis*. In a study of tortoises, 10.3% exhibited chlamydia-like organisms in nasal discharges which seemed to be related to *Cp. pecorum* (Hotzel et al., 2005).

Chlamydiales are mainly associated with respiratory disease, general illness with lethargy, regurgitation, anorexia and may have a high mortality. It is likely that, comparable to the situation in birds, subclinical and persistent carriers exist.

The zoonotic risk of the reptilian Chlamydiales strains is not clear. However, *Cp. pneumoniae* isolates from reptiles have been shown to have a high similarity with the human genotype A (Bodetti et al., 2002). Moreover, the isolation of the *Cp. psittaci* serovar A by Vanrompay et al. (1994) suggests that at least some reptilian isolates could have the potential to cause illness in humans.

Chlamydiales can be detected by taking oral swabs or bronchial lavages for detection of the agent using polymerase chain reaction (PCR), immunofluorescence, ELISA or isolation using cell culture. Treatment is only advised when clinical symptoms are present since the clinical significance of Chlamydiales for reptiles is not clear and the prevalence of Chlamydiales in healthy reptiles has not been assessed. Because of the possible zoonotic risk, the detection of the presence of Chlamydiales in newly acquired reptiles should be taken into consideration.

6.2. Mycobacteriosis

Mycobacteria are classical aetiological agents of granulomatous reactions in human and animal hosts. The genus *Mycobacterium* comprises about 85 species, all sharing the characteristic morphological features of Gram-positive, aerobic, acid–alcohol fast bacteria. The pathogenicity of different mycobacteria varies significantly, and for practical purposes they have been differentiated into two groups: the *Mycobacterium tuberculosis* complex and atypical mycobacteria (Rastogi et al., 2001).

Mycobacterial infections have been reported frequently in a wide variety of reptiles, including snakes, turtles, lizards, and crocodiles. Species isolated from lesions include *M. avium*, *M. chelonae*, *M. fortuitum*, *M. intracellulare*, *M. marinum*, *M. phlei*, *M. smegmatis*, *M. tamnopheos*, and *M. ulcerans* (Aronson, 1929; Olson and Woodard, 1974; Rhodin and Anver, 1977; Brownstein, 1978; Friend and Russel, 1979; Brownstein, 1984; Thoen and Schliesser, 1984; Quesenberry et al., 1986; Frye, 1991; Ariel et al., 1997; Matlova et al., 1998; Hernandez-Divers and Shearer, 2002; Maslow et al., 2002; Greer et al., 2003; Hassl et al., 2004; Schmidt and Reavill, 2005). Reptilian isolates of mycobacteria thus generally belong to MOTT (mycobacteria other than *M. tuberculosis* complex). In the study of Soldati et al. (2004), the incidence of mycobacteria in reptiles with granulomatous lesions was estimated at between 15.6% and 25.6%.

Mycobacterium spp. are ubiquitous in the environment and it is generally believed that they infect animals through defects in the integument or by ingestion. Mycobacterial disease in reptiles is often associated with cutaneous lesions

but systemic illness accompanied by non-specific signs such as anorexia, lethargy and wasting also occurs. At necropsy, greyish-white nodules may be observed in many organs and in the subcutis. Histopathological examination shows typical granulomatous inflammation with multinucleated giant cells, a common feature of these lesions. Unlike mammalian tubercles, calcification has not been observed in reptiles.

The presence of mycobacteria in faeces from clinically healthy reptiles is unlikely to be significant given their ubiquitous environmental presence. Samples for examination should therefore be collected from lesions. Although the ability to obtain a viable *Mycobacterium* culture from a clinical sample would be optimal for the diagnosis of reptilian tuberculosis, many of these mycobacteria have fastidious growth requirements. A fast diagnosis can be facilitated by acid-fast staining of skin scrapings or tissue biopsy. PCR can be used to confirm diagnosis. Soldati et al. (2004) showed that PCR methods are more sensitive than acid-fast staining techniques to diagnose mycobacteria in granulomatous tissues.

Reptilian mycobacteria should be considered as a possible zoonosis (Soldati et al., 2004), although confirmed cases of transfer of mycobacteria from reptiles to humans are rare and the significance for public health is unclear (Hoop, 1997; Hassl et al., 2004). People with immune suppression would be likely to be particularly predisposed.

6.3. Salmonella

All reptiles should be considered positive for *Salmonella* until the contrary has been proven. Reptiles are well known carriers of *Salmonella* spp. Lizards, snakes and tortoises in particular are natural reservoirs for this bacterium. Both *Salmonella enterica* and *S. bongori*, their subspecies and serovars can be found in reptiles (Bäumler et al., 1998). Possible exceptions are some serovars adapted to homeothermic animals such as *S. enterica* subsp. *enterica* serovar Typhi and the species *S. subterranea*.

Clinical salmonellosis, however, is rare in reptiles but might present as salpingitis, dermatitis, osteomyelitis, septicaemia and granulomatous disease. The organism's role in enteric disease in reptiles is not clear but at least in chelonians, and unlike in mammals and birds, *Salmonella* appears to be unable to cross the intestinal barrier in healthy animals (Pasmans et al., 2002, 2003). The importance of *Salmonella* infections is its zoonotic potential and *Salmonella* isolates from reptiles should be considered virulent for humans, possessing virulence factors crucial for the development of gastroenteritis (Pasmans et al., 2005). Indeed, human salmonellosis, associated with pet reptiles is quite common and may account for 5–11% of all human salmonellosis cases in Canada and the United States of America (Woodward et al., 1997; Mermin et al., 2004).

Salmonella zoonoses can be easily avoided by applying basic hygienic measures and through public education (Bradley et al., 1998; de Jong et al., 2005). The risk of contracting salmonellosis from reptiles has led to proposed draconic measures such as a complete ban on the national and international trade of reptiles (Warwick et al., 2001).

Salmonella bacteria can be isolated from cloacal swabs or, preferably, from faeces collected from several animals in the same enclosure over the course of one week. Routine screening for *Salmonella* will often yield at least one *Salmonella* strain with an increased chance of positive results at repeated sampling. Seroconversion, used in other animal species for *Salmonella* diagnosis, appears not to take place in reptiles and serology is thus not an option (Pasmans et al., 2002). These authors do not advise antimicrobial treatment of *Salmonella* infections in reptiles, except for the relatively rare clinical cases. Elimination of *Salmonella* bacteria is difficult to achieve and attempts to treat reptiles with antibiotics, unless monitored carefully and repeatedly, may lead to a false feeling of security. Instead, we advise proper hygienic measures, especially when contact with young, old, pregnant or immunocompromised individuals may occur.

6.4. Dermabacter spp.

Dermabacter-like bacteria cause chronic inflammation of the integument and septicaemia in lizards (Koplos et al., 2000; Pasmans et al., 2004). We have isolated the bacterium from dermatitis lesions and/or the blood of several agamid and iguanid lizards (*Uromastyx* spp., *Agama impalearis*, *Crotaphytus collaris*, *Sauromalus obesus*, *P. vitticeps*, F. Pasmans et al., unpublished data). Dabs (*Uromastyx* spp.) are frequently infected with *Dermabacter*, usually resulting in dermatitis and notably cheylitis (Fig. 7). The disease appears to be highly contagious (probable transmission through direct and indirect contact) and may affect a complete lizard collection within several months. Although the mortality rate in dabs is low and the disease often remains limited to skin infections, in other agamid species such as the desert agama (*A. impalearis*),

Fig. 7. Sahara dab (*Uromastyx geyri*) with cheylitis caused by *Dermabacter*-like bacteria.

Fig. 8. African spurred tortoise (*Geochelone sulcata*) with severe upper respiratory tract disease.

morbidity and mortality can reach 100% (Pasmans et al., 2004).

Thorough clinical examination of newly acquired lizards with special attention to the mouth (cheylitis), cloaca, elbows and knees, and sampling skin lesions for bacteriological examination may help preventing the introduction of this bacterium into a collection. However, animals that have been asymptomatically infected may occur and it is not known whether these carrier animals can be identified. The importance of adequate quarantine measures in these animals cannot be over-emphasised.

6.5. Mycoplasma

Infections with *Mycoplasma* spp. are most often seen in terrestrial tortoises correlated with respiratory signs (upper respiratory tract disease, Fig. 8), and also in crocodiles correlated with pneumonia and polyarthritis (see, for example, Brown et al., 2001).

Upper respiratory tract disease has been found in free living desert tortoises (*Gopherus agassizii*) and is assumed to be one of the causes of the decline in the population (Jacobson et al., 1991). Tortoises show nasal discharge, conjunctivitis and inappetance (Fig. 7) and in severe cases, the bones can be affected. It is a chronic infection which lowers the immune response and allows opportunistic organisms to invade. The infection leads to the production of antibodies which are not able to eliminate the bacterium and do not protect from repeated infection (McLaughlin, 1997). All tortoises which have been in contact with the mycoplasma are considered to be lifelong carriers (Schumacher et al., 1997).

The infection is spread from tortoise to tortoise directly or over short distances. Indirect infection does not seem to play a role as described by McLaughlin (1997). Environmental transmission of naïve tortoises using the burrows of infected and shedding tortoises did not occur but care should nevertheless be taken when handling infected tortoises. Appropriate disinfection of contaminated equipment is recommended. No evidence for vertical transmission has been found (McLaughlin, 1997; Schumacher et al., 1999).

The infective agent has been isolated and used for an experimental infection of tortoises. With this experiment, Koch's Postulates were fulfilled and *Mycoplasma agassizii* was demonstrated as an important respiratory pathogen in the tortoise (Brown et al., 1994). Recently, new pathogenic mycoplasma have been described that are also able to cause disease in tortoises (Brown et al., 2004).

So far, *M. agassizii* has been found in desert tortoises, (*G. agassizii*), gopher tortoises (*Gopherus polyphemus*), red-footed tortoises (*Geochelone carbonaria*), leopard tortoises (*Geochelone pardalis*), Indian starred tortoises (*Geochelone elegans*) and radiated tortoises (Brown et al., 1994). DNA of *M. agassizii* has been found in all species of European terrestrial tortoises and Egyptian tortoises (*T. kleinmanni*), *Geochelone sulcata* and *Indotestudo forstenii* (Blahak et al., 2004; Soares et al., 2004). Leopard tortoises and Russian tortoises in particular show severe symptoms after infection (Blahak et al., 2004). One study has revealed the presence of the agent in free living *Testudo hermanni* in France (Mathes et al., 2001).

The detection of *M. agassizii* in diseased tortoises requires microbial culture, molecular biological investigations and serological methods (Schumacher et al., 1993; Brown et al., 1994, 1995). Culturing of the bacterium is difficult and time consuming (up to 6 weeks). Molecular biological investigations using nested-PCR and restriction enzyme analysis can be performed in 2–3 days and are therefore more suitable for routine diagnostics (Brown et al., 1995). A nasal wash with 1–2 mL of sterile saline is necessary. The sample should be investigated as soon as possible (within 2 days) or otherwise be frozen immediately. Detection of mycoplasma using PCR is also possible using tissue harvested from the nasal cavity or the lungs of dead tortoises.

Investigations for antibodies are performed using a specific ELISA and these are useful in determining the status of a clinically healthy tortoise or to confirm a diagnosis in chronically diseased animals (Schumacher et al., 1997). Correlation of the presence of antibodies with disease has been proven (Schumacher et al., 1993, 1997) but at present the test is not available in Europe.

7. Entry control for viruses in reptiles

7.1. Herpesvirus

Herpesviruses seem to be of major importance in marine turtles and terrestrial tortoises (for example, Rebell et al., 1975; Jacobson et al., 1986; Herbst, 1994; Herbst et al., 1995; Lu et al., 2000).

Herpesvirus infection in terrestrial tortoises was first diagnosed in 1982 and 1985 (Harper et al., 1982; Jacobson

et al., 1985). Typical outbreaks in big collections of terrestrial tortoises were documented in 1989 by a number of different groups of authors (Cooper et al., 1988; Braune et al., 1989; Heldstab and Bestetti, 1989; Lange et al., 1989). Isolation of the virus followed some years later (Biermann and Blahak, 1993; Kabisch and Frost, 1994). Different strains of herpesviruses have been characterized (Biermann, 1995; Marschang et al., 1997; Teifke et al., 2000; Murakami et al., 2001; Marschang et al., 2003; Blahak and Tornede, 2004). The disease has been recognized in a variety of European (Blahak, 2000), North American (Pettan-Brewer et al., 1996), South American (Jacobson et al., 1985) and African tortoises (Oettle et al., 1990).

Clinical symptoms usually start with serous nasal discharge, salivation and loss of appetite (Braune et al., 1989; Lange et al., 1989; Müller et al., 1990; McArthur et al., 2002). Later, thick caseous membranes cover the tongue and pharynx (Fig. 9). Sometimes the oesophagus and lungs are involved. Some tortoises show diarrhoea. Rarely, central nervous symptoms such as walking in circles can be observed. In chronic cases, difficulties in swallowing food are seen.

Sensitive species like Hermann's tortoise (*T. hermanni*) or exotic species of terrestrial tortoises will succumb to the infection within days, whereas more resistant species such as the spur-thighed tortoise (*Testudo graeca*) or marginated tortoise (*Testudo marginata*) will develop antibodies and recover after some weeks (Kabisch and Frost, 1994). The antibodies prevent spread and multiplication of the virus, but will not eliminate it. Thus, every tortoise which has survived a herpesvirus infection and is carrying antibodies against herpesvirus has to be considered a latent carrier of herpesvirus and a threat to a naïve collection, even if the animals look perfectly healthy and active.

If the antibody titre is decreasing due to stress, bad husbandry, illness or a lowered immune system after hibernation, the virus can be reactivated. The tortoise will again start shedding virus with nasal discharge, saliva and faeces and will be able to infect other tortoises. It can take months after introducing a carrier in a collection before shedding starts. The transmission of virus is possible from tortoise to tortoise directly or indirectly with contaminated vessels. At present it is not known whether virus can be transferred to the offspring during the development of the egg. The diagnosis of a suspected herpesvirus infection can be confirmed using virological, serological and molecular biological methods. It is important to choose the adequate method for the case in question.

Fig. 9. Spur thigh tortoise (*Testudo ibera*) with glossitis due to a herpesvirus infection.

In acute cases with clinical symptoms, preparation of oral smears on slides can be stained with haematoxylin–eosin to reveal intranuclear inclusion bodies (Müller et al., 1990). However, the result of this fast test depends on the presence of epithelial cells in the sample and a negative result does not exclude a herpesvirus infection. Oral swabs should be investigated virologically using cell culture or Herpesvirus-PCR. The swabs should be moistened in sterile saline and investigated as soon as possible. If the swabs have to be stored, they should be frozen at −20 or better −70 °C. Different PCR protocols have been published (Vandevanter et al., 1996; Teifke et al., 2000; Murakami et al., 2001) and show different sensitivities for the detection of tortoise herpesvirus strains (Marschang et al., 2003; Blahak and Tornede, 2004). Only the PCR described by Vandevanter et al. (1996) will recognise all herpesvirus strains.

The examination of serum or plasma for the presence of circulating antibodies is not the best way to determine a herpesvirus infection in a tortoise with clinical symptoms. If it is an acute case, the tortoise will start to produce antibodies after some weeks. The result of the determination of the circulating antibody titre in the first weeks after infection will be negative, even if the tortoise has a herpesvirus infection.

Determining the circulating antibody titre is of the utmost importance in preventing a herpesvirus infection transferred by clinically healthy carriers. Every new acquisition should be kept in quarantine with no direct or indirect contact with other tortoises for at least 6 weeks, or, better, several months - including hibernation. During this period of time, tortoises should be tested at least once, or preferably twice with an interval of at least 6 weeks, for antibodies against tortoise herpesvirus. The antibodies are usually determined using a neutralization test in cell culture (Frost and Schmidt, 1997; Marschang et al., 2001). Some publications have shown that serologically different strains exist (Biermann, 1995; Marschang et al., 1997; Blahak, 2000; Marschang et al., 2001; Blahak and Tornede, 2004) and therefore two different herpesvirus strains should be used. Titres >1:8 are considered positive, a titre <1:8 is considered suspect requiring repeat investigations in 4–6 weeks.

PCR from oral swabs taken from clinically healthy tortoises is not recommended. As long as the immune system of the tortoises is active, the virus will stay inactivated and is not excreted so the test will be negative even if the tortoise carries virus in its cells.

7.2. Paramyxoviruses

One of the first virus infections to be documented in reptiles was the paramyxovirus infection of snakes (Ophidian paramyxovirus, OMPV). After an outbreak in Switzerland in 1976 (Fölsch and Leloup, 1976), several reports of this disease were published (Jacobson et al., 1980, 1981; Ahne et al., 1987; Van Horn, 1989; Wells and Bowler, 1989; Ahne and Neubert, 1991; Blahak et al., 1991). The virus has been characterized and placed in a separate group of the family Paramyxoviridae (Kurath et al., 2004).

Venomous snakes of the families Crotalidae and Viperidae seem to be particularly susceptible to this disease (Fölsch and Leloup, 1976; Jacobson et al., 1981; Homer et al., 1995) but the virus has also been found in Colubrids and Boids (Ahne et al., 1987; Ahne and Neubert, 1991; Blahak, 1995; Oros et al., 2001; West et al., 2001). Mortality rates in crotalids are up to 100% (Jacobson and Gaskin, 1989). Symptoms are variable depending on the pathogenicity of the virus strain and on the species infected. In collections of crotalids, sudden death is often noticed without any symptoms. Most snakes show respiratory and sometimes neurological signs. The virus is easily spread by aerosol and contact (Fölsch and Leloup, 1976) so new acquisitions should be kept in a separate room during quarantine. There is no evidence of vertical transmission.

To diagnose paramyxovirus in a collection, necropsy, virological and molecular biological investigations of dead snakes are important. The virus can be isolated most frequently from lung, brain and kidney tissues and also from oral and cloacal swabs. As described above, the swabs should be moistened with sterile saline and investigated as soon as possible. Molecular biological investigations can be carried out as have been described by Ahne et al. (1999), Franke et al. (2001) or Sand et al. (2004). However, as the authors have failed to detect every strain tested, results should be evaluated with caution.

Serology on blood samples is helpful with new acquisitions of unknown status or in cases of convalescent snakes. Two samples should be obtained with an 8-week interval to determine any rise in titre suggesting a recent infection. As there are serological differences between virus strains, two strains should be used (Blahak, 1995; Jacobson et al., 1999). Titres <1:16 (Blahak and Wellen, 1995) or 1:20 (Jacobson et al., 1999) are considered negative, titres >1:16 (Blahak and Wellen, 1995) or 1:80 (Jacobson et al., 1999) are considered positive.

7.3. Inclusion body disease

IBD is a highly prevalent disease in boid snakes. The name stems from the typical histopathological lesions with marked eosinophilic intracytoplasmic inclusions in the tissues. It sporadically occurs in colubrid and viperine snakes but it is not clear whether "IBD" in non-boid snakes has the same aetiology (Raymond et al., 2001; Fleming et al., 2003). Boid species that appear to be most sensitive to IBD are *B. constrictor* and tree boas (*Corallus* spp.), whereas pythons appear to be much less often affected than boas (Garner and Raymond, 2004). IBD is thought to be associated with retrovirus infection but little is known concerning the pathogenesis of the disease.

Blood sucking parasites (e.g. snake mites *O. natricis*) are candidates for the spread of the disease in any collection of snakes (Schumacher et al., 1994). Symptoms include mainly regurgitation, followed by inappetance and progressive nervous symptoms, eventually resulting in the death of the animal (Fig. 10). In some cases, dermatitis, vertebral anomalies and/or neoplastic processes are noted (for a review see Vancraeynest et al., 2006). The disease is always fatal and can cause serious damage to collections. To limit the risk of introducing IBD into a snake collection, we recommend a combination of prolonged quarantine of 6 months, treatment against ectoparasites and the collection of liver biopsies for histological examination. Screening for the presence of IBD can be achieved by taking organ biopsies.

A high number of hepatocytes in liver biopsies from affected snakes generally contain a large eosinophilic intracytoplasmic inclusion, characteristic of the disease (Garner and Raymond, 2004). Alternatively, a biopsy from oesophageal tonsilar tissue can be used (Garner and Raymond, 2004). Although the inclusions are often present in cells in the Jacobson's organ, cytological examination of stained smears from this organ rarely reveals the presence of these inclusions. Blood smears can be used in boas providing a fast and non-invasive tool. Blood smears should be made twice during the quarantine period. Suspect smears can be confirmed using a liver biopsy.

7.4. Adenovirus

Adenoviruses were first detected in 1983 (Heldstab and Bestetti, 1984) and 1984 (Jacobson et al., 1984). Recent investigations have revealed that those viruses belong to the group of atadenoviruses (Wellehan et al., 2004).

Fig. 10. A common boa (*Boa constrictor*) with inclusion body disease showing head tilt and multiple skin lesions.

Adenovirus infections of lizards have become increasingly important and especially in young bearded dragons (*P. vitticeps*) the virus is able to cause illness and sudden death (Julian and Durham, 1985; Jacobson et al., 1996; Boyer and Frye, 2000; Kim et al., 2002; Blahak et al., 2005). Sometimes neurological signs (Kim et al., 2002) or an unusual dark colouring of the skin can be observed (Blahak et al., 2005). Accompanying viruses such as Dependoviruses, Reoviruses or Iridoviruses or parasitic infections, for example coccidia, can often be diagnosed (Jacobson et al., 1996; Kim et al., 2002; Blahak et al., 2005).

All cases of sudden death in collections of Bearded Dragons should be necropsied and investigated for the presence of Adenoviruses, using histological or molecular biological methods. In some cases typically large intranuclear inclusions are seen, especially in the bile ducts of the liver and the epithelium of the intestine. Adenoviral DNA can be found using PCR (Benkö et al., 2002; Wellehan et al., 2004). At present, there is no known report on the usefulness or otherwise of oral or cloacal swabs for determining the presence of adenoviruses but isolation of adenovirus from faeces has been successful (S. Blahak, unpublished data) and could offer a promising way to look for adenoviruses in collections in the future. There are only anecdotal data on the transmission of the virus; horizontal spread can be assumed, but it is not known whether vertical transmission occurs.

Currently, there is no test available to detect the circulating antibody titre. In contrast to snake adenoviruses, which show a clearly visible cytopathic effect in cell culture, lizard adenoviruses cause only minor changes of the cell layer (in VH2, S. Blahak unpublished data). The cytopathic effect of snake adenoviruses can be used to determine antibodies in cell culture in a neutralization test. In lizard adenoviruses, this is more difficult and additional tests have to be carried out.

8. Conclusion

Our knowledge concerning reptile and amphibian diseases has improved greatly over the last two decades. Preventing the introduction of known pathogenic agents should allow the establishment of healthy breeding groups in captivity. However, some main difficulties will have to be overcome in the future: (1) the lack of good diagnostic screening methods (e.g. for IBD); (2) the scarcity of clinical laboratories in which the correct diagnostic tests can be performed; (3) the lack of knowledge about the normal microbiota of most reptilian taxa and (4) the complete lack of vaccines.

References

Ahne, W., Neubert, W.J., 1991. Isolation of paramyxovirus-like agents from Teju (*Callopistes maculatus*) and Python (*Python regius*). In: Proceedings of the 2nd International Symposium of Virology of Lower Vertebrates, Corvallis, Oregon, USA, pp. 203–210.

Ahne, W., Neubert, W.J., Thomsen, I., 1987. Reptilian viruses: isolation of myxovirus-like particles from the snake *Elaphe oxycephala*. Journal of Veterinary Medicine B 34, 607–612.

Ahne, W., Batts, W.N., Kurath, G., Winton, J.R., 1999. Comparative sequence analyses of sixteen reptilian paramyxoviruses. Virus Research 63, 65–74.

Ariel, E., Ladds, P.W., Roberts, B.L., 1997. Mycobacteriosis in young freshwater crocodiles (*Crocodylus johnstoni*). Australian Veterinary Journal 75, 831–833.

Aronson, J.D., 1929. Spontaneous tuberculosis in snakes. Journal of infectious diseases 44, 215–223.

Barnard, S.M., Upton, S.J., 1994. A Veterinary Guide to the Parasites of ReptilesProtozoa, vol. 1. Krieger Publishing Company, Malabar, Florida, USA.

Bäumler, A.J., Tsolis, R.M., Ficht, T.A., Adams, L.G., 1998. Evolution of host adaptation in *Salmonella enterica*. Infection and Immunity 66, 4579–4587.

Benkö, M., Elö, P., Ursu, K., Ahne, W., LaPatra, S.E., Thomson, D., Harrach, B., 2002. First molecular evidence for the existence of distinct fish and snake adenoviruses. Journal of Virology 76 (19), 10056–10059.

Biermann, R.H., 1995. Isolierung und Charakterisierung von Herpesviren bei Landschildkröten. Diss. Vet. Med., Justus-Liebig-Universität Gießen, Germany.

Biermann, R.H., Blahak, S., 1993. First isolation of a herpesvirus from tortoises with diphtheroid-necrotizing stomatitis. In: Proceedings of the 2nd World Congress of Herpetology, Adelaide, Australia, p. 27.

Blahak, S., 1995. Isolation and characterization of paramyxoviruses from snakes and their relationship to avian paramyxoviruses. Journal of Veterinary Medicine B 42, 216–224.

Blahak, S., 2000. Virusinfektionen bei Reptilien. Praktische Tierarzt 81, 92–112.

Blahak, S., Tornede, C., 2004. Comparison of 35 herpesvirus strains from 6 different species of tortoises. In: Proceedings of the 7th International Symposium of Pathology and Medicine of Reptiles and Amphibians, Berlin, Germany, pp. 16–18.

Blahak, S., Wellen, S., 1995. Investigations on the epidemiology of paramyxovirus infections in snakes. In: Proceedings of the 5th International Colloquium on the Pathology of Reptiles and Amphibians, 31.3.–2.4.1995, Alphen aan den Rijn, The Netherlands.

Blahak, S., Göbel, T., Alexander, D.J., Manvell, R., 1991. Some investigations of the occurrence of paramyxoviruses in snakes. In: Proceedings of the 4th International Colloquium on the Pathology of Reptiles and Amphibians 27–29.9.91, Bad Nauheim, Germany, pp. 17–24.

Blahak, S., Brown, D.R., Schumacher, I.M., 2004. *Mycoplasma agassizii* in tortoises in Europe. In: Proceedings of the 7th International Symposium of Pathology and Medicine of Reptiles and Amphibians, Berlin, Germany.

Blahak, S., Benkö, M., Urzu, K., Wiechert, J., Pasmans, F., 2005. Adenovirusinfektionen bei Reptilien – neueste Erkenntnisse, AG ARK, 9.04.05–10.04.05, Hamburg, Germany.

Bodetti, T.J., Jacobson, E., Wan, C., Hafner, L., Pospischil, A., Rose, K., Timms, P., 2002. Molecular evidence to support the expansion of the host range of *Chlamydophila pneumoniae* to include reptiles as well as humans, horses, koalas and amphibians. Systematic and Applied Microbiology 25, 146–152.

Bonner, B., Denver, M., Garner, M., Innis, C., 2001. *Entamoeba invadens*. Journal of Herpetological Medicine and Surgery 11, 17–22.

Bowman, D.D., 2003. Georgis' Parasitology for Veterinarians, eighth ed. W.B. Saunders, Philadelphia, Pennsylvania, USA.

Boyer, T.H., Frye, F.L., 2000. Suspected adenoviral hepatitis transmission from juvenile to adult Bearded dragons *Pogona vitticeps*. In: Proceedings of the 7th Annual Conference of the Association of Reptilian and Amphibian Veterinarians, Reno, Nevada, USA, pp. 69–71.

Bradley, T., Angulo, F.J., Raiti, P., 1998. Association of reptilian and amphibian veterinarians guidelines for reducing risk of transmission of

Salmonella spp. from reptiles to humans. Journal of the American Veterinary Medicine Association 213, 51–52.

Braune, S., Geiss, V., Thiel, W., 1989. Eine neue durch Herpesviren verursachte Erkrankung bei Landschildkröten. Tierärztliche Praxis 17, 416–419.

Brown, M.B., Schumacher, I.M., Klein, P.A., Harris, K., Correll, T., Jacobson, E.R., 1994. *Mycoplasma agassizii* causes upper respiratory tract disease in the desert tortoise. Infection and Immunity 62, 4580–4586.

Brown, D.R., Crenshaw, B.C., McLaughlin, G.S., Schumacher, I.M., McKenna, C.E., Klein, P.A., Jacobson, E.R., Brown, M.B., 1995. Taxonomic analysis of the tortoise mycoplasmas *Mycoplasma agassizii* and *Mycoplasma testudinis* by 16S rRNA gene sequence comparison. International Journal of Systematic Bacteriology 45, 348–350.

Brown, D.R., Nogueira, M.F., Schoeb, T.R., Vliet, K.A., Bennett, R.A., Pye, G.W., Jacobson, E.R., 2001. Pathology of experimental mycoplasmosis in American alligators. Journal of Wildlife Diseases 37, 671–679.

Brown, D.R., Merritt, J.L., Jacobson, E.R., Klein, P.A., Tully, J.G., Brown, M.B., 2004. *Mycoplasma testudineum* sp. nov., from a desert tortoise (*Gopherus agassizii*) with upper respiratory tract disease. International Journal of Systematic Evolutionary Microbiology 54, 1527–1529.

Brownstein, D.G., 1978. Reptilian mycobacteriosis. In: Montali, R.J. (Ed.), Mycobacterial Infections of Zoo Animals. Smithsonian Institution Press, Washington, DC, USA, pp. 265–268.

Brownstein, D.G., 1984. Mycobacteriosis. In: Hoff, G.L., Frye, F.L., Jacobson, E.R. (Eds.), Diseases of Amphibians and Reptiles. Plenum Press, New York, USA, pp. 1–23.

Burridge, M.J., Simmons, L.A., Simbi, B.H., Peter, T.F., Mahan, S.M., 2000. Evidence of *Cowdria ruminantum* infection (heartwater) in *Amblyomma sparsum* ticks found on tortoises imported into Florida. Journal of Parasitology 86, 1135–1136.

Camin, J.H., 1953. Observations on the life history and sensory behavior of the snake mite, *Ophionyssus natricis*, (Gervais) (Acarina: Macronyssidae). The Chicago Academy of Sciences, Special Publication 10, pp. 1–75.

Cooper, J.E., Gschmeissner, S., Bone, R.D., 1988. Herpes-like virus particles in necrotic stomatitis of tortoises. The Veterinary Record 123, 544.

Cranfield, M.R., Graczyk, T.K., 1994. Experimental infection of elaphid snakes with *Cryptosporidium serpentis* (Apicomplexa: Cryptosporidiidae). Journal of Parasitology 80, 823–826.

Cranfield, M.R., Graczyk, T., Wright, K., Frye, F.L., Raphael, B., Garner, M., 1999. Cryptosporidiosis. Bulletin of the Association of Reptilian and Amphibian Veterinarians 9, 15–24.

de Jong, B., Andersson, Y., Ekdahl, K., 2005. Effect of regulation and education on reptile-associated salmonellosis. Emerging Infectious Diseases 11, 398–403.

Eckert, J., Friedhoff, K.T., Zahner, H., Deplazes, P., 2005. Lehrbuch der Parasitologie für die Tiermedizin. Enke Verlag, Stuttgart, Germany.

Everett, K.D.E., Bush, R.M., Andersen, A.A., 1999. Emended description of the order Chlamydiales, proposal of Parachlamydiaceae fam. nov. and Simkaniaceae fam. nov., each containing one monotypic genus revised taxonomy of the family Chlamydiaceae, including a new genus and five new species, and standards for the identification of organisms. International Journal of Systematic Bacteriology 49, 415–440.

Fleming, G.J., Heard, D.J., Jacobson, E.R., Buergelt, C., 2003. Cytoplasmic inclusions in Corn snakes, *Elaphe guttata*, resembling inclusion body disease of Boid snakes. Journal of Herpetological Medicine and Surgery 13, 18–22.

Fölsch, D.W., Leloup, P., 1976. Fatale endemische Infektion in einem Serpentarium. Tierärztliche Praxis 4, 527–536.

Frank, W., 1985. Amphibien und Reptilien. In: Isenbügel, E., Frank, W. (Eds.), Heimtierkrankheiten. Ulmer, Stuttgart, Germany, pp. 161–402.

Franke, J., Essbauer, S., Ahne, W., Blahak, S., 2001. Identification and molecular characterization of 18 paramyxoviruses isolated from snakes. Virus Research 80 (1–2), 67–74.

Friend, S.C., Russel, E.G., 1979. *Mycobacterium intracellulare* infection in a water monitor. Journal of Wildlife Diseases 15, 229–233.

Frost, J.W., Schmidt, A., 1997. Serological evidence of susceptibility of various species of tortoises to infections by herpesvirus. Verhandlungsberichte der Erkrankungen der Zootiere 38, 25–27.

Frye, F.L., 1991. Infectious diseases: fungal, actinomycete, bacterial, rickettsial, and viral diseases. In: Frye, F.L. (Ed.), Biomedical and Surgical Aspects of Captive Reptile Husbandry. Krieger Publishing Company, Malabar, Florida, USA, pp. 101–160.

Garner, M.M., Raymond, J.T., 2004. Methods for diagnosing inclusion body disease in snakes. In: Proceedings of the Association of Reptilian and Amphibian Veterinarians Congress. Naples, USA, pp. 21–25.

Göbel, T., 1990. Ein Beitrag zur Zusammensetzung der aeroben und mikroaeroben Bakterienflora von Rachen und Kloake gesunder Reptilien in Terrarienhaltung, Diss. Vet. Med. Justus-Liebig-Universität Gießen, Germany.

Graczyk, T.K., Cranfield, M.R., 1996. Assessment of the conventional detection of fecal *Cryptosporidium serpentis* oocysts of subclinically infected captive snakes. Veterinary Research 27, 185–192.

Graczyk, T.K., Fayer, R., Cranfield, M.R., 1996. *Cryptosporidium parvum* is not transmissible to fish, amphibians, or reptiles. Journal of Parasitology 82, 748–751.

Graczyk, T.K., Cranfield, M.R., Bostwick, E.F., 1999. Hyperimmune bovine colostrum treatment of moribund Leopard geckos (*Eublepharis macularius*) infected with *Cryptosporidium* sp. Veterinary Research 30, 377–382.

Greer, L., Strandberg, J., Whitaker, B., 2003. *Mycobacterium chelonae* osteoarthritis in a Kemp's Ridley sea turtle (*Lepidochelys kempii*). Journal of Wildlife Diseases 39, 736–741.

Grego, K.F., Gardiner, C.H., Catao-Dias, J.L., 2004. Comparative pathology of parasitic infections in free-ranging and captive pit vipers (*Bothrops jararaca*). The Veterinary Record 154, 559–562.

Guner, E.S., Watanabe, M., Hashimoto, N., Kadosaka, T., Kawamura, Y., Ezaki, T., Kawabata, H., Imai, Y., Kaneda, K., Masuzawa, T., 2004. *Borrelia turcica* sp. nov., isolated from the hard tick *Hyalomma aegyptium* in Turkey. International Journal of Systematic and Evolutionary Microbiology 54, 1649–1652.

Harper, P.A.W., Hammond, D.C., Heuschele, W.P., 1982. A herpesvirus-like agent associated with a pharyngeal abscess in a desert tortoise. Journal of Wildlife Diseases 18, 491–494.

Hassl, A., Benyr, G., Appelt, S., 2000. Freilebende Amöben als opportunistische Darmparasiten von Reptilien. Mitteilungen der Österreichischen Gesellschaft für Tropenmedizin und Parasitologie 22, 49–54.

Hassl, A., Armbuster, C., Filip, R., 2004. *Mycobacterium*-infections in reptiles, a cause of zoonosis? In: Proceeding of Pathology and Medicine of Reptiles and Amphibians, 7th international symposium, Berlin, Germany.

Heine, J., 1982. Eine einfache Nachweismethode für Kryptosporidien im Kot. Zentralblat für Veterinärmedizin B 29, 324–327.

Heldstab, A., Bestetti, G., 1984. Virus associated gastro-intestinal diseases in snakes. Journal of Zoo Animal Medicine 15, 118–128.

Heldstab, A., Bestetti, G., 1989. Herpesviridae causing glossitis and meningoencephalitis in land tortoises (*Testudo hermanni*). Herpetopathologia 1, 5–9.

Herbst, L.H., 1994. Fibropapillomatosis of marine turtles. Annual Review of Fish Diseases 4, 389–425.

Herbst, L.H., Jacobson, E.R., Moretti, R., Brown, T., Sundberg, J.P., Klein, P.A., 1995. Experimental transmission of green turtle fibropapillomatosis using cell-free tumor extracts. Diseases of Aquatic Organisms 22, 1–12.

Hernandez-Divers, S.J., Shearer, D., 2002. Pulmonary mycobacteriosis caused by *Mycobacterium haemophilum* and *M. marinum* in a royal python. Journal of the American Veterinary Medicine Association 220, 1661–1663.

Homer, B.L., Sundberg, J.P., Gaskin, J.M., Schumacher, J., Jacobson, E.R., 1995. Immunoperoxidase detection of ophidian paramyxovirus

in snake lung using a polyclonal antibody. Journal of Veterinary Diagnostic Investigations 7, 72–77.

Hoop, R.K., 1997. Public health implications of exotic pet mycobacteriosis. Seminars in Avian and Exotic Pet Medicine 6, 3–8.

Hotzel, H., Blahak, S., Diller, R., Sachse, K., 2005. Evidence of infection in tortoises by *Chlamydia*-like organisms that are genetically distinct from known Chlamydiaceae species. Veterinary Research Communications 29, 71–80.

Ippen, R., 1992. Ein Überblick über die parasitären Befunde bei Reptilien.- DVG-Tagung FG Kleintierkrankheiten "Grundlagen der Reptlienmedizin", 17.10.1992. Berlin, Germany, pp. 33–40.

Jacobson, E.R., Gaskin, J.M., 1989. Paramyxoviral infections of snakes: An historical overview. In: Proceedings of the third International Colloquium on the Pathology of Reptiles and Amphibians, Florida, USA, pp. 15–16.

Jacobson, E.R., Telford, S.R., 1990. Chlamydial and poxvirus infections of circulating monocytes of a flap-necked chameleon (*Chamaeleo dilepis*). Journal of Wildlife Diseases 26, 572–577.

Jacobson, E.R., Gaskin, J.M., Simpson, C.F., Terrell, T.G., 1980. Paramyxo-like virus infection in a Rock Rattlesnake. Journal of the American Veterinary Medicine Association 177, 796–799.

Jacobson, E.R., Gaskin, J.M., Page, D., Iverson, W.O., Johnson, J.W., 1981. Illness associated with paramyxo-like virus infection in a zoologic collection of snakes. Journal of the American Veterinary Medicine Association 179, 1227–1230.

Jacobson, E.R., Gardiner, C.H., Foggin, C.M., 1984. Adenovirus-like infection in two Nile crocodiles. Journal of the American Veterinary Medicine Association 185, 1421–1422.

Jacobson, E.R., Clubb, S., Gaskin, J.M., Gardiner, C., 1985. *Herpesvirus*-like infection in Argentine tortoises. Journal of the American Veterinary Medicine Association 187, 1227–1229.

Jacobson, E.R., Gaskin, J.M., Roelke, M., Greiner, E.C., Allen, J., 1986. Conjunctivitis, tracheitis, and pneumonia associated with herpesvirus infection in green sea turtles. Journal of the American Veterinary Medicine Association 189, 1020–1023.

Jacobson, E.R., Gaskin, J.M., Mansell, J., 1989. Chlamydial infection in puff adders (*Bitis arietans*). Journal of Zoo Wildlife Medicine 20, 364–369.

Jacobson, E.R., Gaskin, J.M., Brown, M.B., Harris, R.K., Gardiner, C.H., LaPointe, J.L., Adams, H.P., Reggiardo, C., 1991. Chronic upper respiratory tract disease of free-ranging desert tortoises (*Xerobates agassizii*). Journal of Wildlife Diseases 27, 296–316.

Jacobson, E.R., Kopit, W., Kennedy, F.A., Funk, R.S., 1996. Coinfection of a bearded dragon, *Pogona vitticeps*, with adenovirus- and dependovirus-like viruses. Veterinary Pathology 33, 343–346.

Jacobson, E.R., Flanagan, J.P., Rideout, B., Ramsay, E.C., Morris, P., 1999. Ophidian paramyxovirus. Bulletin of the Association of Reptilian and Amphibian Veterinarians 9, 15–18.

Jacobson, E.R., Morris, P., Norton, T.M., Wright, K., 2001. Quarantine. Journal of Herpetological Medicine and Surgery 11, 24–30.

Jacobson, E.R., Heard, D., Andersen, A., 2004. Identification of *Chlamydophila pneumoniae* in an emerald tree boa, *Corallus caninus*. Journal of Veterinary Diagnostic Investigation 16, 153–154.

Julian, A.F., Durham, P.J.K., 1985. Adenoviral hepatitis in a female bearded dragon (*Amphibolurus barbatus*). New Zealand Veterinary Journal 30, 59–60.

Kabisch, D., Frost, J.W., 1994. Isolation of herpesvirus from *Testudo hermanni* and *Agrionemys horsfieldii*. Verhandlungsberichte der Erkrankungen der Zootiere 36, 241–245.

Kahn, C.M., 2005. The Merck Veterinary Manual. Merck & Co., Inc., Whitehouse Station, NJ, USA.

Kaneene, J.B., Taylor, R.F., Sikarskie, J.G., Meyer, T.J., Richter, N.A., 1985. Disease patterns in the Detroit Zoo: a study of reptilian and amphibian populations from 1973 through 1983. Journal of the American Veterinary Medicine Association 187, 1129–1131.

Kenny, M.J., Shaw, S.E., Hillyard, P.D., Forbes, A.B., 2004. Ectoparasite and haemoparasite risks associated with imported exotic reptiles. The Veterinary Record 154, 434–435.

Kim, D.Y., Mitchell, M.A., Bauer, R.W., Poston, R., Cho, D.Y., 2002. An outbreak of adenoviral infection in inland bearded dragons (*Pogona vitticeps*) co-infected with dependovirus and coccidial protozoa (*Isospora* sp.). Journal of Veterinary Diagnostic Investigation 14, 332–334.

Köhler, G., 1996. Krankheiten der Reptilien und Amphibien. Ulmer, Stuttgart, Germany.

Koplos, P., Garner, M., Besser, T., Nordhausen, R., Monaco, R., 2000. Cheilitis in lizards of the genus *Uromastyx* associated with filamentous Gram positive bacterium. In: Proceedings of the Association of Reptilian and Amphibian Veterinarians 2001, pp. 73–75.

Kostka, V.M., Hoffmann, L., Balks, E., Eskens, U., Wimmershof, N., 1997. Review of the literature and investigations on the prevalence and consequences of yeasts in reptiles. The Veterinary Record 140, 282–287.

Kurath, G., Batts, W.N., Ahne, W., Winton, J.R., 2004. Complete genome sequence of Fer-De-Lance virus reveals a novel gene in reptilian paramyxoviruses. Journal of Virology 78, 2045–2056.

Lange, H., Herbst, W., Wiechert, J.M., Schließer, T., 1989. Elektronenmikroskopischer Nachweis von Herpesviren bei einem Massensterben von griechischen Landschildkröten (*Testudo hermanni*) und Vierzehenschildkröten (*Agrionemys horsfieldii*). Tierärztliche Praxis 17, 319–321.

Lu, Y., Wang, Y., Yu, Q., Aguirre, A.A., Balazs, G.H., Nerurkar, V.R., Yanagihara, R., 2000. Detection of herpesviral sequences in tissues of green turtles with fibropapilloma by polymerase chain reaction. Archives of Virology 145, 1885–1893.

Marschang, R.E., Gravendyck, M., Kaleta, E.F., 1997. Herpesviruses in tortoises: Investigations into virus isolation and the treatment of viral stomatitis in *Testudo hermanni* and *T. graeca*. Journal of Veterinary Medicine B 44, 385–394.

Marschang, R.E., Frost, J.W., Gravendyck, M., Kaleta, E.F., 2001. Comparison of 16 chelonid herpesviruses by virus neutralization tests and restriction endonuclease digestion of viral DNA. Journal of Veterinary Medicine B 48, 393–399.

Marschang, R.E., Gleiser, C.B., Roth, B.N., Pfitzner, A.J.P., Böhm, R., 2003. Characterization of 11 different tortoise herpesviruses using PCRs based on three different gene loci. In: Proceedings of the Association of Reptilian and Amphibian Veterinarians, pp.19–20.

Maslow, J.N., Wallace, R., Michaels, M., Foskett, H., Maslow, E.A., Kiehlbauch, J.A., 2002. Outbreak of *Mycobacterium marinum* infection among captive snakes and bullfrogs. Zoo Biology 21, 233–241.

Mathes, K.A., Jacobson, E.R., Blahak, S., Brown, D.R., Schumacher, I.M., Fertard, B., 2001. Mycoplasma and herpesvirus detection in European terrestrial tortoises in France and Morocco. In: Proceedings of the Association of Reptilian and Amphibian Veterinarians, pp. 97–98.

Matlova, L., Fischer, O., Kazda, J., Kaustova, J., Bartl, J., Horvathova, A., Pavlik, I., 1998. The occurrence of mycobacteria in invertebrates and poikilothermic animals and their role in the infections of other animals and man. Veterinarni Medicina 43, 115–132.

McArthur, S., Blahak, S., Kölle, P., Jacobson, E.R., Marschang, R.E., Origgi, F., 2002. Chelonian herpesvirus. Journal of Herpetological Medicine and Surgery 12 (2), 14–31.

McConnachie, E.W., 1955. Studies on *Entamoeba invadens* Rodhain, 1934, *in vitro*, and its relationship to some other species of *Entamoeba*. Parasitology 45, 452–481.

McLaughlin, G.S., 1997. Upper respiratory tract disease in gopher tortoises, *Gopherus polyphemus*: pathology, secondary immune responses, transmission, and implications for conservation and management. PhD dissertation, Department of Wildlife Ecology and Conservation, University of Florida, Gainesville, USA.

Mermin, J., Hutwagner, L., Vugia, D., Shallow, S., Daily, P., Bender, J., Koehler, J., Marcus, R., Angulo, F.J., 2004. Emerging Infections Program FoodNet Working Group. Reptiles, amphibians, and human *Salmonella* infection: a population-based, case-control study. Clinical Infectious Diseases 38, 253–261.

Müller, M., Sachsse, W., Zangger, N., 1990. Herpesvirus-Epidemie bei der Griechischen (*Testudo hermanni*) und der Maurischen Landschildkröte (*Testudo graeca*) in der Schweiz. Schweizer Archiv für Tierheilkunde 132, 199–203.

Murakami, M., Matsuba, C., Une, Y., Nomura, Y., Fujitanin, H., 2001. Development of species-specific PCR techniques for the detection of tortoise herpesvirus. Journal of Veterinary Diagnostic Investigation 13, 513–516.

Oettle, E.E., Steytler, Y.G.M., Williams, M.C., 1990. High mortality in a tortoise colony. South African Journal of Wildlife Research 20, 21–25.

Olson, G.A., Woodard, J.C., 1974. Miliary tuberculosis in a reticulated python. Journal of the American Veterinary Medicine Association 164, 733–735.

Oros, J., Sicilia, J., Torrent, A., Castro, P., Deniz, S., Arencibia, A., Jacobson, E.R., Homer, B.L., 2001. Immunohistochemical detection of ophidian paramyxovirus in snakes in the Canary Islands. The Veterinary Record 149, 21–23.

Pantchev, N., 2005. Parasitosen bei Reptilien (Schlangen, Schildkröten und Echsen). In: Beck, W., Pantchev, N. (Eds.), Praktische Parasitologie bei Heimtieren. Schlütersche Verlagsgesellschaft mbH, Hannover, Germany, pp. 229–288.

Pasmans, F., De Herdt, P., Dewulf, J., Haesebrouck, F., 2002. Pathogenesis of infections with *Salmonella enterica* serovar Muenchen in the turtle *Trachemys scripta scripta*. Veterinary Microbiology 87, 315–325.

Pasmans, F., Van Immerseel, F., Van den Broeck, W., Velge, P., Bottreau, E., Ducatelle, R., Haesebrouck, F., 2003. Interactions of *Salmonella enterica* subsp. *enterica* serovar Muenchen with intestinal explants of the turtle *Trachemys scripta scripta*. Journal of Comparative Pathology 128, 119–126.

Pasmans, F., Martel, A., van Heerden, M., Devriese, L., Decostere, A., Haesebrouck, F., 2004. Dermatitis and septicaemia in a captive population of *Agama impalearis* caused by unknown Actinobacteria. In: Proceedings of the 7th International Symposium of Pathology and Medicine of Reptiles and Amphibians, Berlin, Germany.

Pasmans, F., Martel, A., Boyen, F., Vandekerchove, D., Wybo, I., Van Immerseel, F., Heyndrickx, M., Collard, J.M., Ducatelle, R., Haesebrouck, F., 2005. Characterization of *Salmonella* isolates from captive lizards. Veterinary Microbiology 110, 285–291.

Pettan-Brewer, K.C.B., Drew, M.L., Ramsay, E., Mohr, F.C., Lowenstine, L.J., 1996. Herpesvirus particles associated with oral and respiratory lesions in a California desert tortoise (*Gopherus agassizii*). Journal of Wildlife Diseases 32, 521–526.

Quesenberry, K.E., Jacobson, E.R., Allen, J.L., Cooley, A.J., 1986. Ulcerative stomatitis and subcutaneous granulomas caused by *Mycobacterium chelonei* in a *Boa constrictor*. Journal of the American Veterinary Medicine Association 189, 1131–1132.

Rastogi, N., Legrand, E., Sola, C., 2001. The mycobacteria: an introduction to nomenclature and pathogenesis. Revue Scientifique et Technique de l'Office International des Epizooties 20, 21–54.

Raymond, J.T., Garner, M.M., Nordhausen, R.W., Jacobson, E.R., 2001. A disease resembling inclusion body disease of boid snakes in captive palm vipers (*Botriechis marchi*). Journal of Veterinary Diagnostic Investigation 13, 82–86.

Rebell, G., Rywlin, A., Haines, H., 1975. A herpesvirus-type agent associated with skin lesions of green sea turtles in aquaculture. American Journal of Veterinary Research 36, 1221–1224.

Rhodin, A.G., Anver, M.R., 1977. Mycobacteriosis in turtles: cutaneous and hepatosplenic involvement in a *Phrynops hilari*. Journal of Wildlife Diseases 13, 180–183.

Sand, M.A., Latimer, K.S., Gregory, C.R., Rakich, P.M., Jacobson, E., Pennick, K.E., 2004. Molecular diagnosis of paramyxovirus infection in snakes using reverse transcriptase-polymerase chain reaction and complementary deoxyribonucleic acid: ribonucleic acid in situ hybridization. Journal of Veterinary Diagnostic Investigation 16, 442–448.

Schmidt, R.A., Reavill, D.R., 2005. Survey of reptile diseases. In: Proceedings of the Association of Reptilian and Amphibian Veterinarians. ARAV twelfth annual conference; Tucson, Arizona, USA.

Schultz, H., 1975. Human infestation by *Ophionyssus natricis* snake mite. British Journal of Dermatology 93, 695–697.

Schumacher, I.M., Brown, M.B., Jacobson, E.R., Collins, B.R., Klein, P.A., 1993. Detection of antibodies to a pathogenic mycoplasma in desert tortoises (*Gopherus agassizii*) with upper respiratory tract disease. Journal of Clinical Microbiology 31, 1454–1460.

Schumacher, J., Jacobson, E.R., Homer, B.L., Gaskin, J.M., 1994. Inclusion body disease in boid snakes. Journal of Zoo and Wildlife Medicine 25, 511–524.

Schumacher, I.M., Hardenbrook, D.B., Brown, M.B., Jacobson, E.R., Klein, P.A., 1997. Relationship between clinical signs of upper respiratory tract disease and antibodies to *Mycoplasma agassizii* in desert tortoises from Nevada. Journal of Wildlife Diseases 33, 261–266.

Schumacher, I.M., Rostal, D.C., Yates, R.A., Brown, D.R., Jacobson, E.R., Klein, P.A., 1999. Persistence of maternal antibodies against *Mycoplasma agassizii* in desert tortoise hatchlings. American Journal of Veterinary Research 60, 826–831.

Siroky, P., Kamler, M., Modry, D., 2004. Long-term occurrence of *Hemolivia* cf. *mauritanica* (Apicomplexa:Adeleina:Haemogregarinidae) in captive *Testudo marginata* (Reptilia: Testudinidae): evidence for cyclic merogony? Journal of Parasitology 90, 1391–1393.

Slomka-McFarland, E., 2006. Disinfectants for the vivarium. In: Mader, D.R. (Ed.), Reptile Medicine and Surgery. Saunders, Elsevier, St Louis, Missouri, USA, pp. 1085–1087.

Soares, J.F., Chalker, V.J., Erles, K., Holtby, S., Waters, M., McArthur, S., 2004. Prevalence of *Mycoplasma agassizii* and chelonian herpesvirus in captive tortoises (*Testudo* sp.) in the United Kingdom. Journal of Zoo and Wildlife Medicine 35, 25–33.

Soldati, G., Lu, Z.H., Vaughan, L., Polkinghorne, A., Zimmerman, D.R., Huder, J.B., Pospischil, A., 2004. Detection of mycobacteria and chlamydiae in granulomatous inflammation of reptiles: a retrospective study. Veterinary Pathology 41, 388–397.

Teifke, J.P., Löhr, C.V., Marschang, R.E., Osterrieder, N., Posthaus, H., 2000. Detection of chelonid herpesvirus DNA by nonradiactive in situ hybridization in tissues from tortoises suffering from stomatitis-rhinitis complex in Europe and North America. Veterinary Pathology 37, 377–385.

Thoen, C.O., Schliesser, T.A., 1984. Mycobacterial infections in cold-blooded animals. In: Kubica, G.P., Wayne, L.G. (Eds.), The Mycobacteria: A Sourcebook. Dekker, New York, USA, pp. 1297–1311.

Vancraeynest, D., Pasmans, F., Martel, A., Chiers, K., Meulemans, G., Mast, J., Zwart, P., Ducatelle, R., 2006. Inclusion body disease in boid snakes: a review and description of three cases in boa constrictors in Belgium. The Veterinary Record 158, 757–760.

Vandevanter, D.R., Warrener, P., Bennett, L., Schultz, E.R., Coulter, S., Garber, R.L., Rose, T.M., 1996. Detection and analysis of diverse herpesviral species by consensus primer PCR. Journal of Clinical Microbiology 34, 1666–1671.

Van Horn, G., 1989. An outbreak of paramyxoviral infection in a mixed collection of snakes. In: Proceedings of the third International Colloquium on the Pathology of Reptiles and Amphibians; 13.–15.1.89, Orlando, Florida, USA, p. 19.

Vanrompay, D., De Meurichy, W., Ducatelle, R., Haesebrouck, F., 1994. Pneumonia in Moorish tortoises (*Testudo graeca*) associated with avian serovar A *Chlamydia psittaci*. Veterinary Record 135, 284–285.

Warwick, C., Lambiris, A.J.L., Westwood, D., Steedman, C., 2001. Reptile-related salmonellosis. Journal of the Royal Society of Medicine 94, 124–126.

Wellehan, J.F.X., Johnson, A.J., Harrach, B., Benko, M., Pessier, A.P., Johnson, C.M., Garner, M.M., Childress, A., Jacobson, E.R., 2004. Detection and analysis of six lizard adenoviruses by consensus primer PCR provides further evidence of a reptilian origin for the atadenoviruses. Journal of virology 78 (23), 13366–13369.

Wells, S., Bowler, K., 1989. An outbreak of paramyxovirus in the reptile collection of Audubon Park and Zoological Garden. In: Proceedings

of the third International Colloquium on the Pathology of Reptiles and Amphibians 13.–15.1.89, Orlando, Florida, USA, pp. 1–2.

West, G., Garner, M., Raxmond, J., Latimer, K.S., Nordhausen, R., 2001. Meningoencephalitis in a Boelen's python (*Morelia boeleni*) associated with paramyxovirus infection. Journal of Zoo and Wildlife Medicine 32 (3), 360–365.

Woodward, D.L., Khakhria, R., Johnson, W.M., 1997. Human salmonellosis associated with exotic pets. Journal of Clinical Microbiology 35, 2786–2790.

Xiao, L.H., Ryan, U.M., Graczyk, T.K., Limor, J., Li, L.X., Kombert, M., Junge, R., Sulaiman, I.M., Zhou, L., Arrowood, M.J., Koudela, B., Modry, D., Lal, A.A., 2004. Genetic diversity of *Cryptosporidium* spp. in captive reptiles. Applied and Environmental Microbiology 70, 891–899.

Zwart, P., Vanrompay, D., 2004. *Chlamydophila* infection in small, European viperid snakes. In: Proceedings of Pathology and Medicine of Reptiles and Amphibians, 7th International Symposium. Berlin, Germany.