Possibilities to differentiate wild born from captive bred reptiles

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POSSIBILITIES TO DIFFERENTIATE WILD BORN FROM CAPTIVE BRED REPTILES

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ABSTRACT

A large number of reptiles have been imported to Europe during the last years. Governmental officers are very often challenged by the question, whether these animals are farmed as documented in the papers or taken directly from the wild. The answer has very often to be given by the veterinarian performing the first inspection.

What possibilities does the veterinarian have to distinguish between captive bred and wild caught reptiles?

In 2011 a large number of reptiles was confiscated and housed in the Reptile Rescue Center Munich, to decide whether the majority - freshly imported from Indonesia - were really bred in Indonesia as declared or not. During the first health inspection of 69 green tree pythons (*Morelia viridis*) and 57 monitors of different species (*V. beccari, V. macraei, V. similis, V. prasinus, V. yowonoi, V. kordensis, V. boehmei, V. melinus, V. jobiensis, V. salvator, V. salvadorii, V. indicus, V. reisingeri*) fresh injuries by tick bites and attached living ticks on some animals were found. Furthermore, behavioural patterns were documented during this first period. Additionally faecal samples and blood smears were examined at different labs.

The examination of 58 faecal samples showed the following parasites (relevant groups):

Kapsulotaenia spp. (found in 10 faecal samples; detected in *M. viridis* and *V. reisingeri* - Fig. 1) are proteocephalidean cestodes (Proteocephalidae: Acanthotaeniinae) producing characteristic egg clusters (Chambrier 2006). These parasites are common in boid snakes (*Morelia*) and monitors (*Varanus*; e.g. *K. sandgroundi* or *K. saccifera* among others) in Southeast Asia and Australia. For proteocephalidean cestodes, a life cycle with two intermediate hosts is proposed. E.g. for the genus *Ophiotaenia* (Proteocephalidae: Proteocephaliinae) it is proved for two species parasitizing snakes (*O. europaea* und *O. perspicua*; Thomas 1941, Biserkov and Kostadinova 1997,) that only one intermediate host is not sufficient. First intermediate host are copepods (e.g. *Cyclops* spp.) and the second amphibians. Paratenic (transport) hosts probably also plays a role. In latter case up to three intermediate hosts are involved. In captivity it therefore appears impossible for *Kapsulotaenia* to develop, because they need at least two intermediate hosts, which are furthermore adapted to specific water biotopes. Therefore, the findings of *Kapsulotaenia* in some of the confiscated animals points toward wild caught reptiles.

Spirurids (found in 18 faecal samples; e.g. *Physaloptera* spp. found as an adult in *V. salvadorii* as well as *Physaloptera*-like-eggs in *V. macraei, V. prasinus* and *M. viridis* - Fig. 2) can be found in a wide spectrum of vertebrates (Anderson 2000). Spirurids are usually about 2cm long (however, some species can be over 10cm) and, depending on the species, inhabit the digestive tract (or its walls), various other tissues or serosal membranes. Members of the genus *Physaloptera* e.g. parasitize a lot of lizards and snakes (in the stomach or small intestine) and their eggs always contain a larva at deposition. The larvae develop within a period of 25-30 days in arthropods (commonly insects as beetles, cockroaches, crickets, earwigs among others) as intermediate hosts. Amphibians (e.g. *Rana* frogs) or rodents (e.g. mice) can act also as transport (paratenic) hosts after ingestion of infected intermediate hosts.

A transmission in captivity appears possible in system of e.g. *Phrynosoma* lizards as a final host and ants, which belong to their normal diet, as intermediate host (Frank 1985). But, in boid snakes like *Morelia*, it would be hardly possible. In this case, the first arthropod intermediate is not a part of the normal diet of the snake, and thus, transmission occurs through ingestion of transport (paratenic)
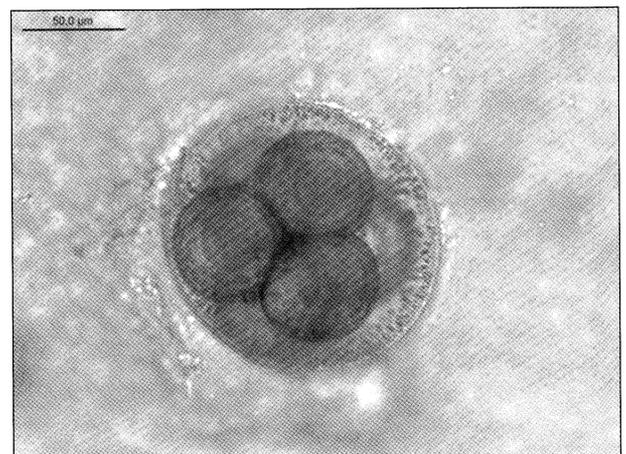
Figure 1 - Characteristic *Kapsulotaenia* egg cluster from *Varanus reisingeri* in a native faecal smear at 630x magnification.
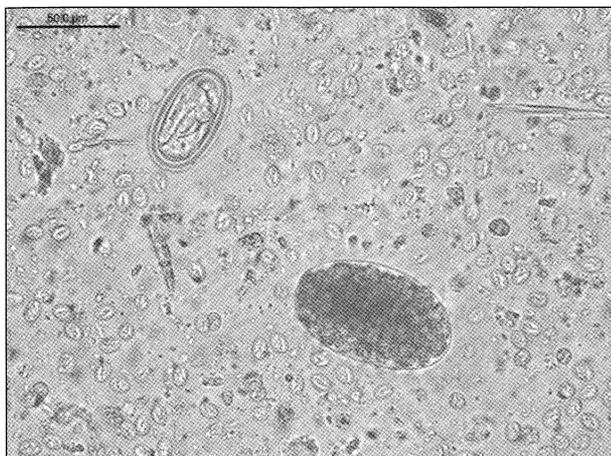
Figure 2 - Spirurid egg (*Physaloptera*-like; close to the scale bar), strongyle-type egg (*Kalicephalus/Herpetostrongylus*-like; farther from the scale bar) as well as numerous coccidian oocysts (*Sarcocystis* sp.) from *Morelia viridis* (400x).
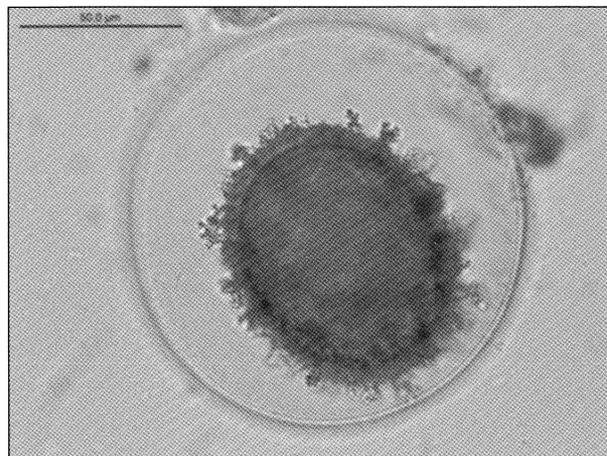
Figure 3 - Pentastomid egg (*Sambonia* sp.) from *Varanus salvadorii* at 630x magnification.

hosts, which themselves ingest the arthropods and harbor the larvae. Thus, in latter case a life cycle with two intermediate hosts is also required (Anderson 2000).

Pentastomids (found in 5 faecal samples; e.g. *Sambonia* spp. eggs found in *V. salvadorii* and *V. reisingeri* - Fig. 3) inhabit in the upper respiratory tract of reptiles, birds and mammals, where they lay eggs. The eggs are coughed out or leave the body of the host through the digestive tract. The eggs are ingested by an intermediate host, which is generally a vertebrate (e.g. various small mammals) in case of Porocephalida members (e.g. with the genus *Sambonia* in monitors) or an arthropod (e.g. cockroach) in Cephalobaenida. The larva hatches in the intermediate host and penetrates the intestine. After that it forms a cyst in the intermediate host's body with a further development to infective nymphs after several moltings. The pentastomid reaches the definitive host when the intermediate host is eaten and crawls from the digestive into the respiratory tract. Although in some cases (monitors in captivity) adult *Sambonia* (in bronchi) and encysted developmental stages (in respiratory mucosa or lever and intestine) were found in a single animal, imitating a direct life cycle through autoinfection, it is believed that the development of *Sambonia* also includes an intermediate host (Bosch 1987; Flach et al., 2000). The simultaneous finding of adults and developmental stages in a single animal is not a proof of a direct life cycle within this animal. Therefore, with pentastomids, an infection in captivity also appears unlikely.

Ascarids (found in 5 faecal samples; e.g. genus *Polydelphis* suspected im *M. viridis* based on egg morphology; Kutzer and Grünberg 1965) are roundworms which can reach a significant length. The life cycle for species infecting boid snakes (e.g. *P. anoura* in *Morelia viridis*; Sprent 1978) is obligatory heteroxenous (indirect) with mainly rodents as intermediate hosts. At least 40 days of development in the intermediate host are necessary for the infectious third stage larva to develop. Only after ingestion of rodents containing the infectious third stage larvae, a development into adult worms in the snake intestine with sub-

sequent egg shedding is possible. The eggs of the ascarids are shed with the faeces of the host. In the environment the larvae develop in the eggs to larvae III. This development lasts, for example, for *Polydelphis quadrangularis* (rattlesnake roundworm) at 25°C about 7 days (Frank 1985).

If the reptiles contained ascarids in the breeding center in Indonesia, they must have had contact to wild rodents or amphibians in their cages or they were fed infected food.

The life cycle of **Capillaria** (eggs detected in 9 faecal samples of *M. viridis*) is generally direct without an intermediate host or indirect with earthworms as intermediate or paratenic host. 53 different species of *Capillaria* have been recorded in reptiles (*Capillaria* sensu latu) (Moravec 2001), most of the hairworms parasitize the stomach and small intestine. But, most life cycles in reptiles are not fully understood. Therefore an indirect development in snakes cannot be excluded.

Trematode eggs were found in 1 faecal samples of *V. reisingeri* (Fig. 4) and in 4 of *M. viridis*. Based of the egg type no species diagnose is possible, but they belong to the class Digenea. It cannot be excluded, that these are species with extraintestinal location, as e.g. *Styphlodora* spp. (Reichenbach-Klinke 1977). Digenetic trematodes have a lifecycle that includes an alternation in generations (sexual-asexual) and obligate host change between the final host (a vertebrate) and one or two intermediate hosts (the first intermediate host is almost always a mollusk; e.g. snail, where the development toward cercaria takes place). Of the many species found in reptiles the cycles of about 50 flukes are known (Frank 1985). The cycle is connected with water or a moist biotope for most species; this is why flukes generally infect animals that live in or near water (aquatic turtles, monitors, crocodilians, some snakes) (Reichenbach-Klinke 1977; Eckert et al., 2008). The most common source of infection in reptiles is certainly via a secondary intermediate host (tadpole, rarely fish, or a few cases, arthropods (such as insects)), which are hosts for the development of the metacercaria. There is no apparent final host specificity, if the second intermediate host is part of
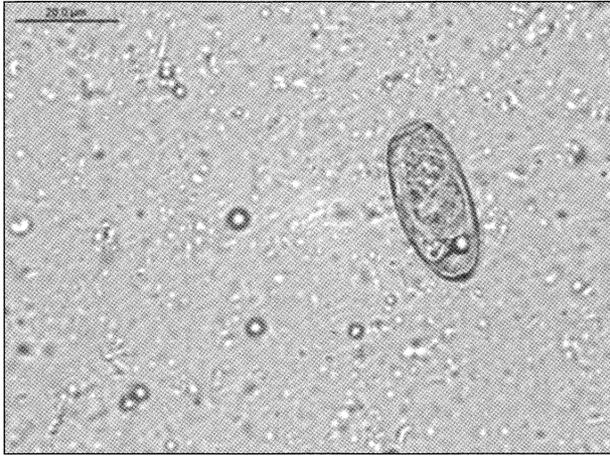
Figure 4 - Digenean trematode egg from *Varanus reisingeri* at 1000x magnification.

the dietary spectrum; but still there is apparently a certain specificity (preference) for the first and second intermediate host. Due to the involvement of multiple (specific) intermediate hosts in the developmental cycle it is highly unlikely, that trematode infection in some of the confiscated animals was acquired in farm conditions.

The examination of blood smears showed an infection with **Hepatozoon spp**. in most of the animals. *Hepatozoon* spp. are often found in wild caught reptiles. *Hepatozoon* (Apixomplexa: Hepatozoidae) has a heteroxenous life cycle and is characterized by the formation of large polysporocystic oocysts occurring in the hemocoel of a definitive host (usually a mosquito; very rare ticks). Various mosquitoes serve as definitive host, including species of *Culex, Aedes*, and *Anopheles*, which are considered to be principal vectors of *Hepatozoon* species from snakes. In the first intermediate host (lizard or frog among others), cystic development occurs mainly in the liver; in the second intermediate host (snake), there are typically 2, or more, rounds of merogony in various internal organs and subsequently development into gametocytes that are found in red blood cells. Among more than 120 species described from snakes, life cycles and host specificity have been studied only in a few cases. Congenital transmission represents another route of infection, as described in the ovoviviparous snake *Nerodia fasciata* (reviewed by Sloboda et al., 2007).

In the first health examination most of the animals showed a severely disturbed general condition. They were in poor nutritional status up to severe emaciation, heavy signs of exsiccosis could be found, some had several acute injuries like lesions and abrasions of the skin and relatively fresh scars. Most animals, especially the monitors and green tree pythons, showed frantic reaction. When they were confronted with human beings, they seemed to be extremely stressed and anxious. Particularly during handling in the enclosures or even at humans stepping into the room a lot of them showed panic reactions. Contrary some species as some of the juvenile monitors like *V. salvadorii* and *V. salvator* were less or not at all impressed by observing human beings.

In the end, those reptiles that showed a physiological behaviour, good indigestion, no signs of tick infection and no parasites with complicated life cycles in the faecal samples were rated as captive bred.

In contrast, the apparently frantic reactions at visual contact to humans and especially the occurrence of parasites having complicated development cycles including different intermediate hosts allowed to classify most of the animals as taken from the wilderness.

The differentiation in this case was hampered by the fact that the animals were declared as captive bred in Indonesia. This means, that some of the parasites occurring naturally in Indonesia will inevitably be found even in captive bread animals, but some of the parasites whose life cycles include intermediate hosts, should not be found in captive bred snakes.

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