Presumptive viral infections in captive populations of *Boa constrictor* in the Czech Republic

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ABSTRACT: The aim of this study was to present outbreaks of infections with presumptive viral aetiology in boid snakes kept in different private collections in the Czech Republic. A total number of 16 boid snakes (*Boa constrictor*) from five private snake collections were included in this study. The patients exhibited neurological depression, dehydration, chronic anorexia, recurrent regurgitation of food, weak muscle tone and stargazing. The blood profile of patients was characterised by extremely low haematocrit. Hyperuricaemia, hypoglycaemia and/or high concentration of phosphorus were measured in some snakes, but not in all patients. All examined faecal samples were negative for the presence of any species of protozoan parasites as well as for eggs of metazoan parasites. Antibodies against the ophidian paramyxovirus in serum samples were determined by a haemagglutination-inhibition assay. This serologic assay was in the suspect range in one snake. Samples of different tissues from snakes that died or were euthanised were collected for necropsy and prepared for a histological examination. Histology results indicated IBD at least in six snakes, basophilic intracytoplasmatic inclusion bodies were detected in the liver, kidneys, lungs, intestine, stomach, hearth, spleen and pancreas of these patients. More specific assays are needed for the clinical diagnosis and control of viral infections in reptilian breeding collections.

Keywords: reptiles; snakes; paramyxovirus; inclusion body disease

Different strains of clinically important viruses have been isolated from all major snake families (Jacobson et al., 1981, 1999; Ahne, 1991; Blahak et al., 1991; Blahak, 1995; Blahak and Wellen, 1995; Pardo et al., 1995; Vix, 1995; Cranfield and Graczyk, 1996; Schumacher, 1996; Boyer et al., 2000). Documented outbreaks of paramyxoviruses (PMV), retroviruses (IBDV), adenoviruses (AV) and other viral infections could be associated with the import of reptiles from Asia and South America to private collections and zoological collections in the USA, UK, Germany and other countries (Jacobson et al., 1981, 1992; Ahne and Neubert, 1991; Blahak et al.,1991; Pardo et al., 1995; Yates, 2004; Wellehan and Johnson, 2005). Patients suffering from paramyxoviruses exhibit symptoms like respiratory distress and sometimes neurological symptoms like weak muscle, opisthotonus (stargazing), head tremor and flaccid paralysis (Folsch and LeLoup, 1976; Jacobson et al., 1981; Schumacher, 1996). Snakes that were infected with retroviruses (IBDV) showed depression, dehydration, paresis, chronic anorexia and regurgitation of food and also neurological symptoms like disorientation, opisthotonus or weak muscle tone (Pardo et al., 1995; Schumacher, 1996; Garner et al., 2000). It is not possible to differentiate these two viral infections according to clinical symptoms. Despite the fact that in most of documented outbreaks, only a few snakes considered at risk were affected, any

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exposed snakes should be considered infected and serve as carriers. These snakes can remain asymptomatic for up to 12 months. It is known that in clinical virology, four steps of Koch's postulates should be demonstrated before determining an agent as being the cause of a disease. It means that the same strain of the virus that has been isolated from patients must be recovered from an experimentally infected host (Jacobson et al., 1999; Kennedy and Greenacre, 2005). Results of such a transmission of ophidian paramyxovirus in rattlesnakes as well as experimental infection of boa constrictors with orthoreovirus were published (Jacobson et al., 1995; Schragen et al., 2004). The diagnosis of viral infections in snakes and other reptiles is done only in a few specialized laboratories (Blahak, 2000; Van Leeuwaarden et al., 2002). The number of well documented studies about the incidence and prevalence of different viral infections in private and professional collections of snakes in Europe is therefore still limited (Folsch and LeLoup, 1976; Blahak et al., 1991; Blahak and Wellen, 1995; Franke et al., 2001; Marschang et al., 2003). The aim of this study was to present the documented outbreaks of infections with presumptive viral aetiology in boid snakes kept in different private collections in the Czech Republic.

MATERIAL AND METHODS

A total number of 16 boid snakes (*Boa constrictor*) that originated from five private snake collections were included in this study (Table 1).

Collection 1

A four-years-old male boa constrictor (No. 330/03) with cachexia and stargazing was submitted for clinical examination with the history of recurrent regurgitation and weak muscle tone. Blood samples were taken for haematology and plasma biochemistry, fresh fluid contents of stomach and cloaca were examined for the presence of protozoan parasites (*Cryptosporidium* sp.). Adult mites

Table 1. Boa constrictors – patients included in this study (n = 16)

Snake	Sex (F/M)	Age (years)	Weight (kg)	Parasitology	Symptoms	Endoscopy
330/03	M	4	3.03	negative	stargazing, weak muscle tone	–
30/04	F	6	6.00	negative	stargazing (Figure 1)	+
31/04	M	6	3.36	negative	stargazing, dehydration, weak muscle tone, lethargy	+
32/04	F	7	5.20	negative	stargazing, dehydration, weak muscle tone, lethargy	+
33/04	M	0.5	0.53	n.d.	dysecdysis	–
34/04	F	3	2.50	n.d.	dysecdysis	–
35/04	M	5	3.07	n.d.	dysecdysis	–
36/04	F	5	6.22	negative	stargazing, dehydration, weak muscle tone, lethargy	+
117/04	F	2	1.05	negative	stargazing, lethargy, weak muscle tone, dehydration, oedema near the cloaca	–
293/04	M	0.2	0.06	negative	chronic regurgitation, dehydration, meteorism and diarrhoea	–
29/05	F	8	7.61	negative	died during transport	–
30/05	M	5	6.78	negative	stargazing, lethargy	–
31/05	M	5	7.90	n.d.	permanent bilateral mydriasis	–
45/05	F	5	9.73	n.d.	–	–
46/05	F	8	17.70	n.d.	–	–
64/05	M	9	1.34	negative	weak muscle tone, lethargy, chronic rhinitis and stomatitis	+

n.d. – not determined

Figure 1. Stargazing symptom in a *Boa constrictor* with IBDV infection

Ophionyssus natricis were detected in this snake by a previous check at a veterinary clinic, and effective treatment was realised with fipronil (Frontline Spray, Merial, France).

Collection 2

Four snakes with chronic anorexia and neurological symptoms were submitted for a check. Snake No. 30/04 was a six-years-old female boa constrictor suffering from chronic anorexia and stargazing (Figure 1). A six-years-old male (No. 31/04), seven-years-old female (No. 32/04) and five-years-old female (No. 36/04) were all lethargic, deeply dehydrated, with extremely weak muscle tone and stargazing. The keeper had a large private collection with different species of boid snakes and the main reason for submitting these patients to the clinic was a request for a humane form of euthanasia (T61 I.C., Intervet, Netherlands). Another group of three boid snakes, a six-months-old male (No. 33/04), three-years-old female (No. 34/04) and five-years-old male (No. 35/04) suffering from dysecdysis, was included in this study because they were kept together with the previous group.

Collection 3

A two-years-old female boa constrictor (No. 117/04), suffering from chronic anorexia and stargazing, was lethargic, deeply dehydrated, with extremely weak muscle tone. This snake was presented with the problem of regurgitation and oedema of the body in the region localised 15 cm up from the cloacal opening. The eggs of *Ophionyssus natricis* were observed as a contamination of faecal samples. The treatment protocol consisted of enrofloxacin (10 mg/kg S.C q 24 h, Baytril 2.5% inj., Bayer HealthCare, Germany). The patient died on the seventh day of treatment. The other patient from the same collection was a two-months-old male (No. 293/04) suffering from regurgitation, dehydration, meteorism and diarrhoea for one month. Blood samples were taken, faecal and gastric fluid samples were collected by flushing with water and examined for the presence of protozoan parasites. This snake died on the third day of hospitalisation.

Collection 4

A nine-years-old male boa constrictor (No. 64/05), originating from a very large collection of boid snakes, with the history of chronic rhinitis and stomatitis, weak muscle tone and lethargy was submitted for clinical examination and humane form of euthanasia. Blood samples were taken for haematology and plasma biochemistry, fresh contents of cloaca were examined for the presence of parasites.

Collection 5

Another group of patients (Nos. 29–31/05) comprised one eight-years-old female and two five-

years-old males suffering from chronic anorexia. Within the last six months the owner realised the treatment of snakes against mites with organophosphate spray (Arpalit 1% Spray). The males were examined immediately after arrival whereas the female died during transport. Despite their good body condition, both the males had a weak muscle tone. Severe stargazing and lethargy were present in one male whereas the other one showed permanent bilateral paralytic mydriasis. Blood samples were collected into tubes containing heparin. Fresh faecal samples collected by cloacal flushing with water were examined for the presence of protozoan parasites. The last group of snakes (Nos. 45–46/05) comprised two females (five-years- and eight-years-old, respectively) that did not show any symptoms of health problems. These snakes were submitted only for a preventive check because they shared the same room with the previous group in a private collection. Blood samples were taken for haematology, plasma chemistry and serology.

Haematology, plasma chemistry, parasitology

Blood samples (1.5–3 ml) were collected from the ventral coccygeal vein approximately at a third of the tail length from the cloaca with 23G × 1 Luer needles into tubes containing heparin (Heparin Leciva inj., Prague). Blood smears were prepared immediately following the sample collection. The blood smears were air-dried and stained by the Pappenheim method (May-Grünwald + Giemsa-Romanowski). The total erythrocyte and leukocyte counts (RBC, WBC) were determined manually with a haemocytometer with Natt and Herrick's solution; packed cell volume (PCV) was measured by the microhaematocrit method. Haemoglobin concentration (Hb) was determined spectrophotometrically by a standard cyanmethaemoglobin method with one modification: the samples were centrifuged following the red cell lysis to remove nuclear and cytoplasmic debris. The leukocyte differential counts were analyzed with an Olympus BX 51TF light microscope and documented with an Olympus C 3030 digital camera. Plasma biochemistry assays were performed within 2 h after venipuncture by the use of automated analyzers. The concentrations of total protein (TP), glucose, uric acid, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and phosphorus (P) were determined with a CobasMira analyzer (Roche).

Faecal and stomach flushing samples were examined for the presence of protozoan parasites (*Cryptosporidium* sp., *Acanthamoeba* sp., *Entamoeba* sp.) and eggs of metazoan parasites by standard methods that were published previously (Barnard and Upton, 1994).

OPMV antibodies detection in serum samples

Antibodies against the ophidian paramyxovirus in serum samples were determined by a modified haemagglutination-inhibition assay according to Blahak and Wellen (1995). The assay was performed using a suspension of 0.5% chicken erythrocytes. Briefly, the snake sera were adsorbed for one hour with a suspension of 50% chicken erythrocytes to exclude false positive reactions and heated shortly before the assay for 30 min at 56°C. Two different strains of snake paramyxovirus (strain 1356 and 5688) were used. The strains were adjusted to four haemagglutinating units before the start of the test. The test was done with serial doubling dilutions of the sera ($\log_2$) in phosphate buffered saline (0.025 ml of sera and 0.025 ml of PBS). An amount of 0.025 ml virus suspension was added to each well and incubated for 30 minutes. Then 0.05 ml of 0.5% suspension of chicken erythrocytes in PBS was added. The test was read off after 30 minutes. The highest dilution of the serum causing the inhibition of haemagglutination was considered the titre of the serum. A titre of 1:16 and higher was considered positive.

Endoscopy

In five snakes a direct endoscopic examination of lungs with access through the air sac was carried out by the method that was published recently (Jekl and Knotek, 2006). The snake was intubated with an endotracheal tube and kept under general anaesthesia with isoflurane. After an aseptic preparation of the surgery area, a short incision was made in the skin on the right side of the body, at 35–45% of the patient's length, parallelly with the horizontal body axis. Then a blunt perforation of the muscle layer and peritoneum was made. The endoscope with an examination sheath was

introduced through a small incision of the air sac between the two absorbable sutures. Endoscopy was performed using rigid endoscopes (ø 2.7 mm, 18 cm, Hopkins Forward-Oblique Telescope Karl Storz Tuttlingen, ø 4.0 mm, 30 cm, Wolf Tuttlingen) with an examination sheath, endoscopic camera (Endovision Telekam SL, Karl Storz, Tuttlingen) and a xenon light source (Xenon Nova Karl Storz Tuttlingen). After the air-ways examination, the air sac wall, the muscle layer and the skin were closed with three separate sutures.

Post-mortem examination

Samples of tissues (lungs, heart, liver – Figure 2, kidneys, intestine, stomach, pancreas) from seven snakes that died or were euthanised were collected for necropsy, fixed in formalin and prepared for a routine histological examination.

RESULTS

Haematology, plasma chemistry, parasitology

The blood profile of snakes included in this study was characterised by a broad range of detected values except the hematocrit that was extremely low (PCV 0.05–0.36 l/l). Hyperuricaemia, hypoglycaemia and/or high concentration of phosphorus were measured in some snakes, but not in all patients. The results of haematological and plasma chemistry examinations are summarised in Table 2.

All examined faecal samples were negative for the presence of any species of protozoan parasites as well as for eggs of metazoan parasites.

OPMV antibodies detection in serum samples

One snake (No. 36/04) tested for antibodies against paramyxoviruses showed a titre of 1:4 with one of the paramyxovirus strains (5688). All other serum samples were negative.

Intracytoplasmatic inclusion bodies

We did not find any inclusions in erythrocytes or leucocytes. The basophilic intracytoplasmatic inclusion bodies were detected in different tissue samples – in liver (Figure 3), kidneys (Figure 4), lungs, intestine, stomach (Figure 5 and 6), heart, spleen and pancreas in six snakes (Table 3).

DISCUSSION

Parasitic mites and ticks represent a pathogenic risk for boid snakes, and the range of infections carried by them has been well documented (Barnard and Durden, 2000; Boyer et al., 2000; Kenny et al., 2004). The common snake mites (*Ophionissus*

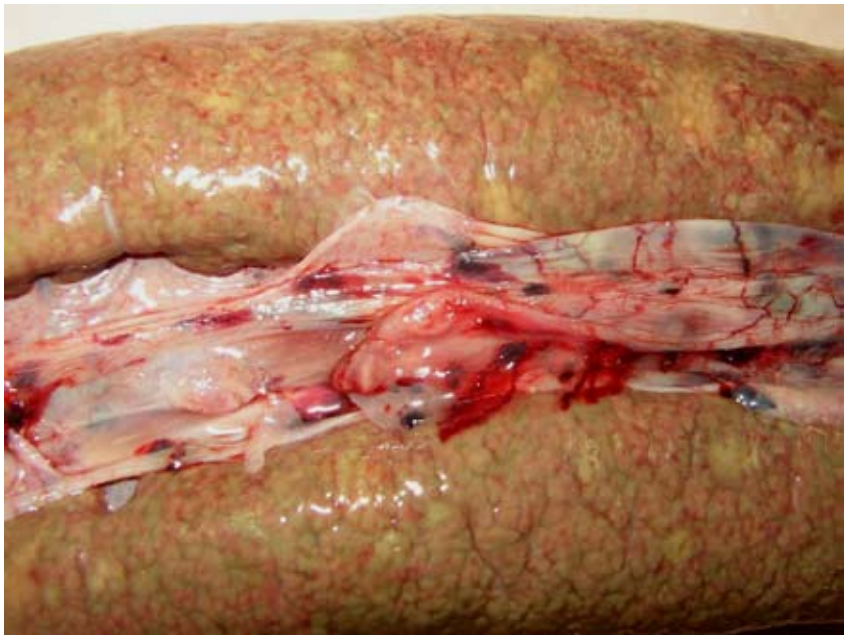

Figure 2. Hepatodystrophy in a *Boa constrictor* with IBDV infection

Table 2. Haematology and plasma chemistry profiles in boid snakes (*Boa constrictor*), *n* = 14*

Values	*Boa constrictor*													
	330/03	30/04	31/04	32/04	33/04	34/04	35/04	36/04	117/04	30/05	31/05	45/05	46/05	64/05
Hb (g/l)	74	41	87	55	–	–	64	61	–	87	84	61	89	63
PCV (l/l)	0.16	0.16	0.36	0.20	0.15	0.05	0.27	0.26	0.23	0.29	0.28	0.18	0.30	0.20
RBC (10^{12}/l)	0.51	0.59	0.86	0.69	0.60	0.12	0.87	0.81	0.64	0.73	0.66	0.46	0.64	0.89
WBC (10^9/l)	15.00	5.25	11.50	11.00	15.50	4.00	8.50	7.25	23.25	11.50	5.50	4.00	18.50	15.50
Lymphocytes (10^9/l)	1.20	0.42	0	1.54	11.03	3.28	1.87	0.94	12.32	2.99	0.71	1.40	10.55	1.55
Monocytes (10^9/l)	1.50	0	0.12	0.11	0	0.12	0.09	0.65	0	0	0.06	0	0	0.78
Heterophils (10^9/l)	6.75	1.20	5.29	3.41	2.84	0.24	0.34	2.18	3.00	6.30	1.30	1.24	5.00	3.57
Azurophils (10^9/l)	5.55	3.52	6.23	5.94	2.05	0.28	3.57	3.26	5.58	1.84	2.86	1.04	2.04	8.53
Basophils (10^9/l)	0	0.16	0.12	0	0	0	0.17	0.15	2.33	0.23	0.61	0.32	0.56	0.31
Eosinophils (10^9/l)	0	0	0	0	0	0.08	0	0.07	0	0.12	0	0	0.37	0.78
Total protein (g/l)	66.9	n.d.	n.d.	n.d.	36.6	75.4	61.2	n.d.	61.2	67.9	89.9	54.4	77.4	n.d.
Glucose (mmol/l)	0.66	n.d.	n.d.	n.d.	1.15	1.13	2.65	n.d.	1.91	2.30	2.52	1.30	2.19	n.d.
Uric acid (μmol/l)	660.7	n.d.	n.d.	n.d.	316.6	244.6	404.5	n.d.	951.6	147.1	712.2	203.9	620.2	n.d.
ALP (μkat/l)	8.66	n.d.	n.d.	n.d.	1.79	0.43	6.16	n.d.	6.55	3.81	4.73	3.02	5.46	n.d.
AST (μkat/l)	1.10	n.d.	n.d.	n.d.	0.09	0.18	0.07	n.d.	0.90	0.42	0.13	0.05	0.21	n.d.
ALT (μkat/l)	0.64	n.d.	n.d.	n.d.	< xx	< xx	0.12	n.d.	0.29	0.18	0.22	0.14	0.57	n.d.
Phosphorus (mmol/l)	–	n.d.	n.d.	n.d.	–	–	1.51	n.d.	2.64	1.44	1.57	1.69	1.58	n.d.

*Female No. 29/05 was not included because she died before a clinical examination. No blood samples were collected from snake No. 293/04; n.d. – not determined

Table 3. Detection of intracytoplasmatic inclusion bodies in *Boa constrictors* (*n* = 7)

Snake	Inclusions	
	erythrocytes/leucocytes	tissue samples
30/04	no inclusion bodies	intracytoplasmatic inclusion bodies in liver, kidney, pancreas and stomach
36/04	no inclusion bodies	no inclusion bodies
63/04	no inclusion bodies	intracytoplasmatic inclusion bodies in liver, kidneys, pancreas, stomach
117/04	no inclusion bodies	intracytoplasmatic inclusion bodies in liver, kidneys, stomach, pancreas
293/04	no inclusion bodies	intracytoplasmatic inclusion bodies in gastric epithelium, pancreas and liver
29/05	no inclusion bodies	intracytoplasmatic inclusion bodies in liver and urethras
64/05	no inclusion bodies	intracytoplasmatic inclusion bodies in liver, pancreas, epithelial cells of stomach, kidneys and lungs

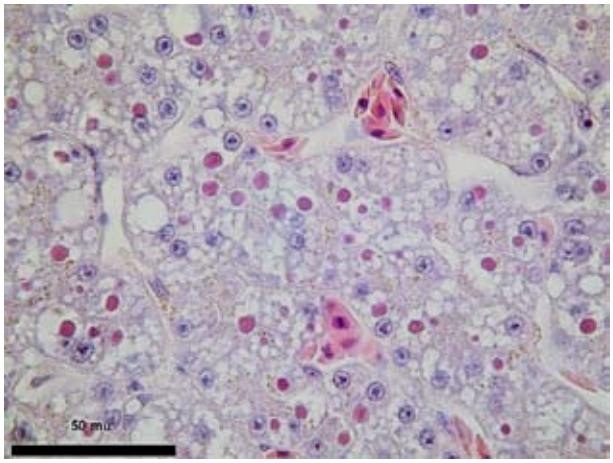

Figure 3. Basophilic intracytoplasmatic inclusion bodies (liver), HE

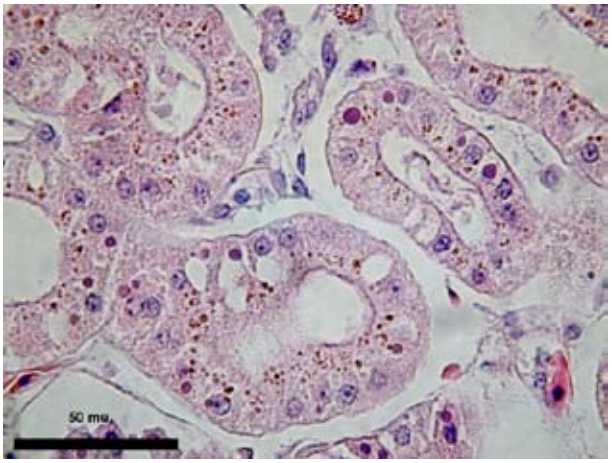

Figure 4. Basophilic intracytoplasmatic inclusion bodies (kidneys), HE

natricis) were present in each of the snake collections that were examined within this study. The potential role of parasitic mites as vectors of viral infections in these collections is therefore suggested.

A number of independent laboratory investigations have been focused mainly on the development of high sensitive and high specific diagnostic assays (HI, VN, ELISA, PCR; Gaskin et al., 1989; Blahak, 1995, 2000; Kania et al., 2000; Franke et al., 2001; Van Leeuwaarden et al., 2002; Yates, 2004; Wellehan and Johnson, 2005). Antibodies against the ophidian paramyxovirus in serum samples of snakes in this study were determined by a haemagglutination-inhibition assay. One patient showed a weak reaction with one paramyxovirus strain. With this weak reaction in one of the investigated snakes, a paramyxovirus infection as cause of the disease in this collection seems to be unlikely. However, virological investigations were not possible due to the lack of material and therefore the paramyxovirus infection cannot be ruled out completely. All other serum samples were negative for OPMV.

Anorexia, dehydration, regurgitation and different symptoms of the central nervous system disease are present in snakes suffering from IBDV infection (Pardo et al., 1995; Jacobson et al., 1999; Garner et al., 2000). This is in accordance with the prevailing clinical symptoms that were observed in the majority of boid snakes included in this study.

However, these are non-specific symptoms and may be caused by different aetiological agents. The presence of inclusion bodies in the organs is in accordance with results of Schumacher (1996). The basophilic intracytoplasmatic inclusion bodies were detected in liver, kidneys, lungs, intestine, stomach, heart, spleen and pancreas in six patients.

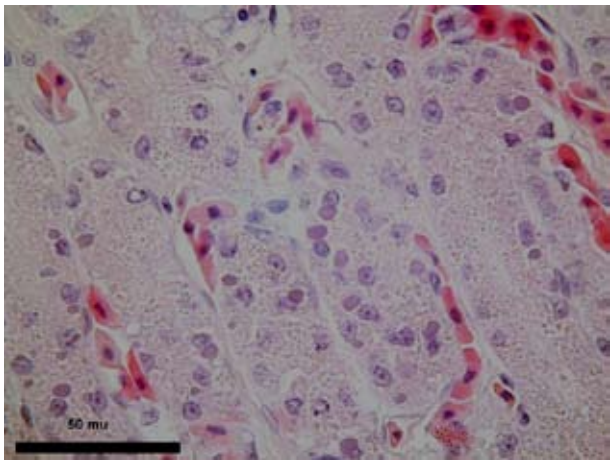

Figure 5. Basophilic intracytoplasmatic inclusion bodies (stomach), HE

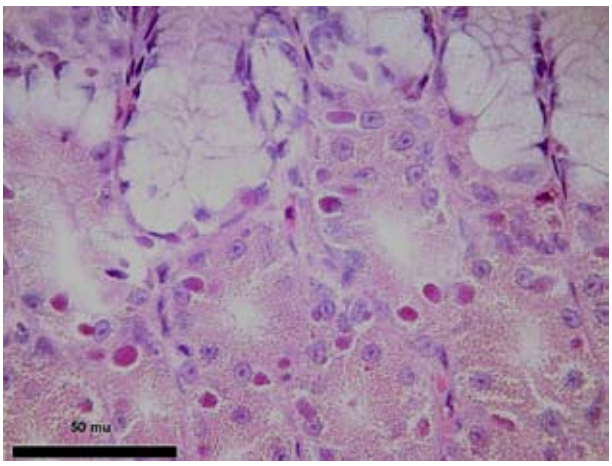

Figure 6. Basophilic intracytoplasmatic inclusion bodies (stomach), HE

In one of the previous well documented outbreaks of viral disease in snakes most of the patients suffered from gout (Blahak et al., 1991). Therefore it is not surprising that high concentrations of uric acid were observed in the plasma of five snakes in the present trial, however a possible influence of viral disease on the kidney function in boid snakes has to be confirmed.

Despite the fact that no specific virological investigation was realised, based on the presence of inclusions and HI assay, six snakes of this study at least should be considered as IBDV positive patients. The identification of paramyxovirus positive snakes can be done by using an HI assay for the screening of antibodies or by investigating oral swabs with PCR or by virus isolation in a cell culture. IBD-positive snakes can be found by the screening of blood smears for inclusion bodies or by examining biopsies of liver or oesophageal tonsils, which is a more reliable but invasive technique. Unfortunately, at the moment there is no other method for diagnosing IBD in living animals.

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