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Short communication

Treatment of cryptosporidiosis in captive green iguanas (*Iguana iguana)*

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ABSTRACT

There are no standard guidelines for the treatment of cryptosporidiosis in reptiles. The aim of this study was to evaluate the efficacy of two cryptosporidiosis therapies in captive green iguanas. Eight green iguanas aged 2–6 years, including 6 (1 ♂ and 5 ♀) animals with chronic diarrhea, received treatment for cryptosporidiosis. The presence of *Cryptosporidium* sp. oocysts was determined in 8 iguanas (100%), *Isospora* sp. oocysts were detected in 3 animals (37.5%), and Oxyuridae eggs were observed in 5 iguanas (62.5%). The animals were divided into two therapeutic groups (A and B). Group A iguanas were administered halofuginone (Halocur, 0,50 mg/ml Intervet Productions S.A., France) at a dose of 110 mg/kg body weight (BW) every 7 days for 5 weeks. Group B animals were administered sulfadiazine and trimethoprim (Norodine Vet Oral Paste sulfadiazine 288,3 mg/g, trimethoprim 58 mg/g, ScanVet Animal Health A/S, Denmark) at 75 mg/kg BW *per os* every 5 days for 5 weeks and spiramycin and metronidazole (Stomorgyl, spiramycin 1500000 IU, metronidazole 250 mg, Merial, France) at 200 mg/kg BW every 5 days for 5 weeks. Both groups received hyperimmune bovine colostrum and subcutaneous fluids. Before treatment, the average number of *Cryptosporidium* sp. oocysts in 1 g of feces was determined at $1.71 * 10^5$ (± 313,262.44) in group A and $1.56 * 10^5$ (± 262,908.53) in group B; the average number of *Isospora* sp. oocysts was determined at $3.53 * 10^3$ (± 1747.38), and the average number of Oxyuridae eggs was determined at 810 (± 496.74). Blood tests were performed once before treatment. The results of blood morphology and biochemistry tests before treatment revealed leukocytosis with a significant increase in heterophile and monocyte counts in all animals. Dehydration, elevated hematocrit values and low levels of Na^+, Ca^{2+}, PO_4^- and Cl^- ions were observed in 6 iguanas. Two iguanas died during treatment. The gross necropsy revealed acute inflammation of gastric and duodenal mucosa, mucosal ecchymoses in the gastrointestinal tract, hepatomegaly and liver congestion, cholecystitis, enlarged kidneys and renal edema and congestion, cystitis, and an absence of fat bodies. Parasites were not detected in any developmental form after 40 days of therapy and during an monthly 18-month follow-up period. Effective treatment of cryptosporidiosis in reptiles minimizes the adverse consequences of disease, improves the animals' well-being and decreases euthanasia rates.

1. Introduction

The growing popularity of North and South American reptiles and amphibians as pets in Central Europe has contributed to the breeding and sale of captive animals. An increase in the prevalence of invasive diseases affecting exotic pets has been reported in veterinary practice (Ras-Norynska and Sokol, 2015). The green iguana (*Iguana iguana)*, a lizard of the family Iguanidae, is frequently admitted to veterinary clinics with symptoms of gastrointestinal and excretory system diseases. The digestive tract of green iguanas is composed of small bowel loops, which pass into the large intestine with a small pouch-like anterior colon that resembles the cecum. Cellulose and other plant foods are digested in the anterior colon. The proximal colon contains two transverse valves: a circular valve and a semilunar valve (Iverson, 1980). Cellulose from plant foods is digested by gut commensal flora. In the diet of these reptiles one should remember about calcium supplementation. Its deficiencies can lead to imbalance with the other nutrients in the body, and can cause muscles and nerves dysfunctions. It is also important during the breeding season in females. Its deficiency can lead to Metobolic Bone disorder (MBD). The proper nutritional requirements ratio of calcium to phosphorus is about 2:1. Changes in the composition of gut microbiota lead to digestive tract dysfunctions that may be exacerbated by parasites (Harp et al., 1992; Berrilli et al., 2012). Cryptosporidia are particularly dangerous for iguanas compared to other parasites. Cryptosporidiosis can be the reason to euthanize an animal. (Xiao et al., 2004; Mitchell and Tully, 2008). Cryptosporidia produce oocysts that are resistant to many environmental stressors. Oocysts are spread by food, mainly water, air and direct contact. Young,

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old and immunosuppressed individuals are particularly susceptible to cryptosporidiosis (Gałęcki and Sokół, 2015). Cryptosporidia are homoxenous parasites. Around 80% of cryptosporidium oocysts are surrounded by a thick wall and are excreted with feces. The remaining oocysts are surrounded by a thin wall which ruptures in the intestines and leads to autoinvasion. Sporulated oocysts contain 4 sporozoites, and oocysts excreted with feces are invasive. There are no standard guidelines for the treatment of cryptosporidiosis in reptiles (Mitchell and Tully, 2008). The aim of this study was to evaluate the efficacy of two cryptosporidiosis therapies in captive-bred green iguanas.

2. Materials and methods

Eight green iguanas (2 ♂ and 6 ♀) aged 2–6 years, including 6 (1 ♂ and 5 ♀) animals with watery and later bloody (in 3 patients III ♀, IV ♀, I ♂) chronic diarrhea, with undigested food in the feces. In 6 subjects were observed following symptoms: anorexia, highly lethargic, muscle wasting, and severely darkened scales. In 2 green iguanas (I ♀, IV ♀) vomiting occurred. The animals showed signs of severe dehydration such as: a large amount of dry sticky mucus in the mouth; wrinkles, lack of flexibility and dry skin; elongation of palatal vessel filling; sunken eyeballs. The animals did not have any infectious diseases before. Lizards were admitted to the Veterinary Clinic of the University of Warmia and Mazury in Olsztyn, Poland. The reptiles were kept in specialist terrariums, where the area for one individual was about 3 m^3, the temperature in the terrarium 29–33 °C, heat islands 38 °C (measured by a elecronic thermometer), humidity 80%. In the terrariums, fluorescent lamps corresponding to the reptile needs of Repti Glo 2.0 and 5.0 (Exo Terra) replaced every 6 months, the light day was 12 h. The iguanas diet consisted of the leaves of wild and cultivated plants (80%), and fruits and vegetables (20%). Before admission, the animals had been treated with amoxicillin and clavulanic acid, but symptoms of diarrhea had intensified. Pathogenic bacterial strains were not detected in a bacteriological examination. All iguanas were subjected to clinical evaluations before treatment. Blood was sampled 1/3 down from the cloaca on the ventral midline from the caudal vein for morphological and biochemical analyses of plasma, which were conducted using Neubauer hemocytometer and Unopette® Eosinophil Determination for Manual Methods stain (Becton Dickinson and Company, Franklin Lakes, New Jork) with the methods described in the Manual of Exotic Pet Practice (Mitchell and Tully, 2008). reference intervals were obtained from "Diseases of *Iguana iguana*" (Anderson, 1992). On the basis of clinical symptoms and hematocrit, percentage of dehydration was determined. Individual cloacal swabs were collected, stained by the Ziehl-Neelsen technique and observed under a light microscope at 200x and 1000x magnification. After diagnosis, every animal was kept in a separate terrarium. Before treatment individual 4 g fecal samples were collected, then homogenized, and examined by the Fülleborn's flotation method with Sheather's solution (454 g granulated sugar, 355 ml tap water and 6 ml full-strength (37%) formaldehyde; solution's specific gravity-1.27). Samples were centrifuged at 3500*g* for 10 min. Each centrifuged sample was used to prepare 3 specimens for the analysis under a light microscope. Test results were the average of three repetitions. Parasites in various developmental forms were identified with the use of the Diagnostic Parasitology for Veterinary Technicians atlas (Hendrix and Robinson, 2011). Parasitological analyses (flotation method) of fecal samples were repeated after 7, 14, 21 and 40 days. After recovering, parasitological studies from three subsequent defecations were performed with monthly interval. The number of *Cryptosporidium* sp. oocysts was counted in the Fuchs-Rosenthal chamber. The number of *Isospora* sp. oocysts in 1 g of stool (OPG) and *Oxyuris* sp. eggs in 1 g of stool (EPG) was determined by flotation in the McMaster chamber (Anon, 1987). The animals' body weights and health condition were monitored once monthly during and after the treatment (18 months).

The treated iguanas were divided randomly into 2 groups (A and B) of 4 individuals each. Group A comprised 3 ♀ (2, 3 and 6 years) and 1 ♂ (6 years), and group B comprised 3 ♀ (3, 6 and 6 years) and 1 ♂ (2 years). Group A animals received halofuginone (Halocur, 0,50 mg/ml Intervet Productions S.A., France) at 110 mg/kg BW every 7 days for 5 weeks. Group B animals received sulfadiazine and trimethoprim (Norodine Vet Oral Paste sulfadiazine 288,3 mg/g, trimethoprim 58 mg/g, ScanVet Animal Health A/S, Denmark) at 75 mg/kg BW *per os* every 5 days for 5 weeks and spiramycin and metronidazole (Stomorgyl, spiramycin 1500000 IU, metronidazole 250 mg, Merial, France) at 200 mg/kg BW every 5 days for 5 weeks. Both groups received hyperimmune bovine colostrum (HIBC) *per os* and subcutaneous saline solution with Ringer's solution, 5% glucose and Duphylate in a 4:4:4:1 ratio (298 mOsmol/l), at 25 ml/kg BW. HIBC was obtained with methods described by Fayer et al. (1989) using *C. parvum*. Pinworm invasion was treated with fenbendazole (Fenbendazol, 100 mg/ml, aniMedica, Germany) administered *per os* at 25 mg/kg BW daily for 7 days. If Oxyuridae eggs were detected in successive analyses of fecal samples, the fenbendazole dose was increased to 75 mg/kg BW every 7 days until a negative result was obtained. Invasion caused by *Isospora* sp. was treated with toltrazuril (Procox, toltrazuril 0,9 mg/ml, emodepside 18 mg/ml, Bayer, Germany) administered *per os* in two doses of 10 mg/kg BW each at a 7-day interval. Terraria were disinfected with 10% ammonia (Fayer et al., 1996). After the treatment supplementation with calcium and vitamin D_3 (Repticalcium with D3, Zoomed, USA) was administered *per os* of 20 mg/kg with food at a 3-day interval.

3. Results

Cryptosporidium sp. oocysts were detected in the fecal smears of all animals; therefore, additional fecal samples were analyzed with the Rapid Cryptosporidium Ag test (VetExpert, BioNote, Inc., Republic of Korea), based on a chromatographic immunoassay for the qualitative detection of *Cryptosporidium* spp. antigens, confirmed the presence of *Cryptosporidium* sp. in 6 (75%) out of the 8 examined iguanas. The test was ineffective in individuals with high *Isospora* spp. OPG (V♀, I ♂). Due to similar progression of disease and identical morphological structure of oocysts observed under a microscope, all animals were diagnosed with cryptosporidiosis, despite the fact that the applied test was not specific for reptiles (bovine test).

Oxyuridae eggs were also detected in 5 iguanas (63%) (3 from group A and 2 from group B) at EPG $\bar{x}$ 810 (± 496.74), and *Isospora* sp. oocysts were detected in 3 animals (38%) (2 from group A and 1 from group B) at OPG $\bar{x}$ $3.53 * 10^3$ (± 1747.38) (Table 1).

The results of blood analyses revealed leukocytosis ($\bar{x}$ 12699.5/µl, ± 2614.61) and a significant increase in heterophile ($\bar{x}$ 75.5%, ± 3.93) and monocyte ($\bar{x}$ 7%, ± 1.13) counts in all animals. Dehydration (10–15%), elevated hematocrit values ($\bar{x}$ 38.38%, ± 6.12), and low levels of Na^+ ($\bar{x}$ 126.88 mmol/l, ± 16,25), Ca^{2+} ($\bar{x}$ 1.8875 mmol/l, ± 0.65), PO_4^- ($\bar{x}$ 1.55875 mmol/l, ± 0.25) and Cl^- ($\bar{x}$ 82 mmol/l, ± 16.20) ions were observed in 6 iguanas presenting clinical symptoms of the disease (Table 2).

Two iguanas died during treatment (25%), including ♀ No. IV from group A on day 8 and ♂ No. I from group B on day 16. The dead individuals were in poor clinical condition and were characterized by the highest OPG values in the first analysis of fecal samples (♀ No. IV OPG $5.5 * 10^5$; ♂ No. I OPG $6.4 * 10^5$). A post-mortem examination revealed severe dehydration, acute inflammation of gastric and duodenal mucosa, mucosal ecchymoses in the gastrointestinal tract, hepatomegaly and liver congestion, cholecystitis, enlarged kidneys and renal edema and congestion, cystitis, and an absence of fat bodies in both animals. Inflammation and enlargement of the ovaries, pelvic congestion and embryonic development arrested in the yolk sac stage were noted in the female. Impresion-smear and acid fast staining from the mucosa of stomach, intestines, gallbladders, urinary tracts and oviducts revealed the presence of *Cryptosporidium* spp. Fecal samples were collected from the rectum post-mortem. Both fecal samples contained a