CLINICAL SIGNS, MANAGEMENT, AND OUTCOME OF PRESUMPTIVE IVERMECTIN OVERDOSE IN A GROUP OF DENDROBATID FROGS

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ABSTRACT

Ivermectin is routinely used as an anthelmintic in amphibians at the National Aquarium, Baltimore. Presumptive inadvertent overdose occurred in dendrobatid frogs administered oral ivermectin via micropipette at volumes below the 0.5 ul lower-limit of the unit. Forty-eight frogs, 19 *Dendrobates auratus* (green and black poison dart frog) and 29 *D. tinctorius* (dyeing and blue poison dart frog), were administered ivermectin as part of a parasite management program. Within 48 hours of administration, clinical signs consistent with ivermectin toxicity developed, including ataxia, flaccid paralysis, pulmonary respiratory depression, and total unresponsiveness to stimuli. Multiple frogs also demonstrated generalized fluid accumulation consistent with hydrocoelom. Clinical signs developed in 31 frogs; 19/19 *D. auratus* and 12/29 *D. tinctorius*. Management included fluid and nutritional support and calcium, antibiotic, and furosemide administration. The overall survival rate for frogs that developed clinical signs was 32% (10/31). Of the 21 mortalities, seven frogs were euthanized and 14 were found dead. In surviving frogs, clinical signs lasted up to three weeks, but were mild after two weeks. Even frogs with severe central nervous system depression survived and supportive care is warranted in toxicity events.

Key words: Amphibian, anuran, *Dendrobates auratus, Dendrobates tinctorius*, ivermectin, toxicity.

INTRODUCTION

Ivermectin is a macrocyclic lactone antiparasitic agent. It is a gamma amino butyric acid (GABA) agonist which binds to glutamate-gated chloride channel receptors. In invertebrates, GABA receptors are located in peripheral neuromuscular junctions and activation of this neuroinhibitory pathway leads to flaccid paralysis (Merck, 2012). In vertebrates, GABA receptors are found primarily in the central nervous system (CNS) though evidence of presence of GABAergic signaling in other locations exists (Aller *et al.*,1997; Hollis and Boyd, 2003; Li *et al.*, 2012; Merck, 2012; Trailović and Nedelijković, 2010). In mammals, ivermectin concentration in the central nervous system is limited due to permeability glycoproten (p-glycoprotein) in the blood-brain barrier (Merola and Eubig, 2012; Roder and Stair, 1998).

Ivermectin is routinely administered by a variety of routes including PO, SC, IM, and topically and recommended anti-nematode dosages are generally 0.2 – 0.4 mg/kg (Plumb, 2011). Despite a generally wide margin of safety, toxicity from overdose or at expected therapeutic dosing has been reported in vertebrate animals including amphibians, cats, dogs, equids, fruit bats, and reptiles (DeMarco *et al.*, 2002; Hautekeete *et al.*, 1998; Hopper *et al.*, 2002; Kenny *et al.*, 2008; Letcher and Glade,1992; Merola and Eubig, 2012; Plummer *et al.*, 2006; Pritchard, 2010; Sladky et al., 2000; Swor *et al.*, 2009; Széll *et al.*, 2001; Teare and Busch, 1983). Signs of toxicity include ataxia, flaccid paralysis, mydriasis, lack of response to stimuli, and, rarely, seizures (Letcher and Glade, 1992; Plumb, 2011; Teare and Busch, 1983; Roder and Stair, 1998). Clinical signs develop within hours to days of administration (Plumb, 2011; Roder and Stair, 1998). A diagnosis of toxicity in veterinary patients is generally made based on history of administration and clinical signs; blood or tissue levels can be evaluated but are not often utilized clinically

(Plumb, 2011; Roder and Stair, 1998). In some cases, clinical signs after administration may be due to systemic reaction to parasite die-off rather than ivermectin toxicosis (Plumb, 2011; Sladky *et al*., 2000; Széll *et al*., 2001). In dogs, a p-glycoprotein deficiency due to ABCB1-1Δ gene mutation leads to CSF drug accumulation and toxicosis at standard dosages (Merola and Eubig, 2012; Wright *et al*., 2011). Changes to the blood brain barrier secondary to inflammation, infection, other medications, or dietary items have been speculated to disrupt the blood-brain barrier and cause toxicosis at standard dosages (Swor *et al*., 2009).

There is no direct antidote to ivermectin. Appropriate supportive and nursing care is provided including cardiorespiratory, fluid, and nutritional support and management to prevent pneumonia and cutaneous pressure sores (Gwaltney-Brant and Meadows, 2012; Hopper *et al*., 2002; Plumb, 2011; Teare and Busch, 1983). Activated charcoal is frequently administered to reduce enterohepatic circulation of the drug (Hooper *et al*., 2002; Roder and Stair, 1998). Intravenous lipid emulsion administration may reduce clinical signs and treatment length but are not universally effective or part of current standard treatment recommendations (Clarke *et al*., 2011; Gwaltney-Brant and Meadows, 2012; Wright *et al*., 2011). Other medications that are periodically utilized but not part of standard treatment recommendations include the GABA antagonist picrotoxin and the short-acting anticholinesterase physostigmine (Hopper *et al*., 2002; Plumb, 2011; Roder and Stair, 1998; Teare and Busch, 1983). Benzodiazepine antagonists such as flumazenil may reduce CNS depression through reversal of ivermectin binding to benzodiazepine binding sites on the GABA receptor (Trailović and Nedelijković, 2010). The prognosis for ivermectin toxicosis is good if adequate supportive care can be provided.

Ivermectin administration has been reported in amphibians and recommended dosages include: 0.2 – 0.4 mg/kg PO, SC, IM, topically; 2 mg/kg topically; and 10 mg/L as a 60 min bath (Letcher and Glade, 1992; Poynton and Whitaker, 2001; Sladky *et al*., 2000; Wiley *et al*., 2009; Wright and DeVoe, 2012; Wright and Whitaker, 2001). In one study, ivermectin dosages of 2 mg/kg topically, 0.2 mg/kg IM, and 0.4 mg/kg IM were safe in leopard frogs (*Rana pipiens*) but dosages of 2 mg/kg IM and 20mg/kg IM were fatal (Letcher and Glade, 1992). Frogs had flaccid paralysis within 10 min to 3 hr of treatment and the majority died within 24 hr. The most common dosage at the National Aquarium, Baltimore is 0.2 mg/kg PO (Clayton, unpublished data).

This case describes clinical signs, management, and outcomes in two dendrobatid species administered a presumed overdose of ivermectin orally.

CASE REPORT

On June 16 and 17, 2009, forty-eight frogs were administered oral ivermectin; 19 *Dendrobates auratus* (green and black poison dart frog) and 29 *D. tinctorius* comprised of 13 azureus (blue poison dark frog) and 16 tinctorius (dyeing poison dark frog) types (Wollenberg *et al*., 2006). Frogs were manually restrained and swabbed for chytrid fungus (*Batrachochytrium dendrobatidis*) testing, administered ivermectin, and moved to clean enclosures with new substrate as part of routine management. Forty-five frogs were captive bred at the facility; three *D. tinctorius* were wild-caught. Frogs were individually identified by skin color patterns (Nelson, 2012) and were considered healthy at the time of treatment.

The *D. auratus* age range was 13- to 65-mo-old and the weight range was 2.25 - 4.66 g. The *D. tinctorius* azureus type age range was 14- to 114-mo-old and weight range was 3.6 – 5.32 g. *D. tinctorius* tinctorius type age range was 10- to 153-mo-old and weight range was 4.37 – 9.12 g (Table 1). Age was measured from the point at which the individual became a terrestrial froglet or estimated age of one year old at time of acquisition in wild-caught frogs.

Frogs were housed in an off-exhibit space. Room temperature was 25 – 28 ˚C (78 – 81 ˚F) with 35 – 70% relative humidity. Frogs were managed in groups of 2 - 4 in glass terrariums following previous published descriptions (Barnett *et al*., 2001). Automatic (four times daily) and manual (once daily) terrarium misting provided increased enclosure micro-climate humidity. All water was carbon filtered fresh water (FFW). Artificial terrarium lighting was provided with two bulbs (ZooMed Laboratories, ReptiSun 5.0 UVB fluorescent bulb; San Luis Obispo, CA) placed 15.2 – 30.5 cm (6 – 12”) above each enclosure on a 12-hour photoperiod.

Frogs were fed fruit flies (*Drosophila melanogaster* and *D. hydei*) or newly hatched house crickets (*Acheta domestica*) three days a week. Fruit flies were raised in-house on fruit fly media (Formula 4-24 Instant *Drosophila* Medium; Carolina Biological, Burlington, NC). Crickets were raised in-house and gut-loaded with a commercial high-protein cricket diet (Hi-Cal Cricket Monster Diet; Zielger Bros. Inc., Gardners, PA) before being fed out. All insects were dusted with an oyster-shell based calcium supplement (Repti Calcium without D3; Zoo Med Laboratories, San Luis Obipso, CA) twice weekly and a mineral supplement with vitamin D_3 once weekly (Miner-All Indoor; Sticky Tongue Farms, Sun City, CA).

Ivermectin (Endo-Mectin, 1%, Aspen Veterinary Resources, Liberty, MO) was administered orally via a 0.5 – 10 ul micropipette (Eppendorf Reference10, Eppendorf AG, Hamburg, Germany) to 19 *D. auratus* and 11 *D. tinctorius* azureus type. The following day, 2 *D. tinctorius* azureus types and 16 *D. tinctorius* tinctorius types were treated. The intended dose was 0.2 mg/kg and the drug was not diluted.

Clinical signs began 24 - 48 hr after ivermectin administration. Major clinical signs included ataxia, reduced or absent reflexes, including righting reflex, reduced or absent buccal pumping, and complete lack of response to noxious stimuli. The neuroanatomical localization was generalized lower motor and diffuse cerebral. Hydrocoelom was significant in some frogs between 48 and 96 hr post-administration. Differential diagnosis included ivermectin toxicity, toxicity from another source such as the new substrate or disinfectants used in cage cleaning, and acute infection. Ivermectin toxicity was considered the likely diagnosis due to history of drug administration, clinical signs, and lack of clinical signs in hundreds of other frogs handled identically but without ivermectin administration.

A scoring system was used to categorize case status in individual animals: (0) no clinical signs; (1) mild ataxia, normal spontaneous movement present; (2) moderate ataxia, reduced spontaneous movement but reaction to light tactile stimuli; (3) severe ataxia, reduced spontaneous movement but reaction to moderate tactile stimuli, righting reflex moderately impaired; (4) maintains partially upright position, no spontaneous movement or movement in reaction to tactile stimuli, righting reflex significantly impaired; (5) unable to maintain upright position, no reaction to noxious stimuli, complete flaccid paralysis, no righting reflex.

Fluid/electrolyte support was started within 48 hr of signs developing and ambulatory frogs were given access to both FFW and reptile ringer's solution (RR) composed of 50% Lactated ringer's solution (Veterinary lactated ringer's solution injection USP, Abbott Laboratories, North Chicago, IL) and 50% 0.45% saline with 2.5% dextrose (2.5% Dextrose and 0.45% Sodium chloride injection USP, Abbott Laboratories, North Chicago, IL). Non-ambulatory frogs were soaked for various periods (3 hr to constant) in water or RR with the oral cavity and nostrils clear of the fluid or placed on saturated unbleached paper towels. Frogs with significant hydrocoelom received furosemide (Salix 5%; Intervet Inc., Millsboro, DE) 2 mg/kg PO; furosemide was administered if frogs had 0.5 g weight gain from weight on day of ivermectin administration. Furosemide was administered to eleven frogs between days 2 and 4.

Additional supportive care was started on day 6 for frogs with clinical signs and continued for two consecutive days after clinical signs resolved. Frogs received enrofloxacin (Baytril, 22.7%; Bayer HealthCare LLC, Shawnee Mission, KS) 10 mg/kg PO SID, and calcium (calcium glycerophosphate 5mg/ml and calcium lactate 5mg/ml compounded injectable solution; BCP Veterinary Pharmacy, Houston, TX) 5mg/kg topically SID. Nutritional support was provided with a commercial amphibian and reptile food powder (Amphibian and Carnivorous Reptile Gel #5MEO; Mazuri PMI Nutrition International LLC, Richmond, IN) mixed with room temperature FFW at 1:4 ratio given at 30ml/kg body weight PO SID.

Thirty-one frogs developed clinical signs; 19/19 *D. auratus* and 12/29 *D. tinctorius* comprised of 12/13 azureus types and 0/16 tinctorius types. The overall survival rate for frogs showing clinical

signs was 32% (10/31). The survival rate for *D. auratus* showing clinical signs was 32% (6/19) and for *D. tinctorius* azureus types was 33% (4/12).

At 24 hr post-administration, nine frogs presented with a score of 3 or less, 13 presented with a score of 4, and nine presented with a score of 5 (Table 2). Frogs that presented with a score of 3 or less had a 67% survival rate. Frogs that presented with a score of 4 had a 31% survival rate (Table 2). No frogs that presented with a score of 5 survived (Table 2).

Multiple frogs declined and attained a higher maximum score than presenting score (Table 3, Figure 1). Frogs with a maximum score of 3 or less had a 100% survival rate but the majority of frogs reached a maximum score of 4 or 5 (Table 3). Frogs with a maximum score of 4 had a 50% survival rate and frogs with a maximum score of 5 had a 15% survival rate (Table 3).

Of the 10 frogs that developed clinical signs and survived, nine were clinically normal and off treatment on day 15 and the remaining frog was normal and off treatment on day 21 (Figure 1). Presence of significant hydrocoelom did not obviously impact case outcome; 40% (4/10) of frogs that survived and 33% (7/21) of mortalities were administered furosemide.

Twenty-one frogs died; fourteen were found dead and seven were euthanized with an overdose of buffered tricaine methanesulfonate (Finquel; Argent Chemical Laboratories, Redmond, WA) as a topical bath. The first mortality occurred on day 1, 19 mortalities occurred days 6 to 9, and the last mortality occurred on day 11. Frogs had typically been at a score of 5 for 24 hr or more when found dead or euthanized.

Gross necropsy was completed on 15 of the 21 mortalities. The most common findings were skin sloughing and distention of the gastrointestinal tract. Cytology of sloughed skin showed no evidence of *Batrachochytrium dendrobatidis* infection. Four frogs were submitted for histology to the Department of Molecular and Comparative Pathology, Johns Hopkins University, School of Medicine. No consistent histologic lesions were identified and ivermectin toxicity was considered the likely cause of death. The liver of one frog had an ivermectin concentration of 9.5 ppm determined at the Animal Disease Lab, Illinois Department of Agriculture. A sample of ivermectin from the same bottle used to treat the frogs diluted 1:10 with sterile water to create a total volume of 1 ml was also submitted and the level was 1,105.5 ppm (1.1055 mg/ml).

Two statistical approaches were used to evaluate the relation between weight and mortality. A logistic regression was performed using all frogs for which the sex was known (n = 36). The response variable utilized was mortality, a binary, categorical variable, and the effect of weight, controlling for age and sex, was assessed. Weight had a significant effect on survival outcome, controlling for age and sex (Table 4). In addition, probit regression was used to assess the main effect of weight on mortality (averaged over the covariates and sex) and the estimated weight at which the probability of death was 0.50 (LW50) was calculated. Relationship of weight to species (averaged over the covariates and sex) was assessed with analysis of variance. Probit regression demonstrated a significant inverse relation between weight and mortality (slope = -0.71, $p < 0.0004$, estimated LW50 = 4.08 g). There was a relation of weight to species. The mean weight of *D. auratus* was 3.1 g, *D. tinctorius* azureus 4.5g, and *D. tinctorius* tinctorius 6.3g and the differences were statistically significant ($p < 0.0001$).

DISCUSSION

Dendrobates tintorius and *D. azureus* were initially considered separate species due to morphological difference; molecular testing has subsequently classified them as the single species *D. tinctorius* (Wollenberg *et al.*, 2006). At the time of this case, they were inventoried as separate species at the National Aquarium, Baltimore and case information has been presented using the identifying convention of type to indicate the different groups.

Clinical signs in these frogs were consistent with ivermectin toxicosis (Letcher and Glade, 1992; Plumb, 2011; Teare and Busch, 1983; Roder and Stair, 1998). In addition, hydrocoelom developed in some animals and furosemide was administered to 11 frogs. Furosemide appears to be clinically effective in dendrobatids (Clancy *et al.*, 2011), presumably increasing fluid clearance utilizing a pathway different from diuresis through the loop of Henle (Plumb, 2011). Presence of fluid did not obviously impact case outcome and was transitory in the first four days post-administration.

Exposure to toxins from handling and cage change is unlikely as hundreds of other frogs had identical exposures without showing clinical signs. Massive parasite die-off could also cause similar signs but no evidence of high parasite loads was present on gross necropsy or histology. Primary infectious disease seems unlikely as clinical signs were only seen in animals administered ivermectin. Ivermectin was present in the liver of one animal tested and supports systemic absorption of the drug via this administration method. Further tissue studies were not accomplished in this case, but may be helpful in evaluating other cases of proposed toxicity.

Ivermectin toxicity is the most likely differential based on history of drug administration, clinical signs and course of disease, and ancillary testing.

Ivermectin has been used extensively at the National Aquarium, Baltimore for decades with no toxicity events appreciated, including in over 500 dendrobatids comprised of these and other species (Clayton, unpublished data). The standard protocol (SP) was to administer 0.2 mg/kg PO or occasionally topically using a micropipette accurate between 0.5 – 10 ul. An injectable cattle product at 10 mg/ml was diluted with sterile water or 0.9% saline to 1 mg/ml or 0.1 mg/ml to achieve appropriate concentrations for micropipette volume measurement. Using the SP, frogs 2.0– 4.9 g would have received 4.0 - 9.8 ul of 0.1 mg/ml solution and frogs between 5.0 – 10.0 g would have received 1.0 – 2.0 ul of 1mg/ml solution. Micropipettes are professionally serviced every 6 - 12 months as part of an equipment quality control program.

In this case, overdose likely occurred due to a change in administration technique. Undiluted ivermectin was administered via micropipette set to a volume measurement below the 0.5 ul lower-limit. Calculated volumes using undiluted ivermectin were 0.04 – 0.18 ul. The micropipette dial could be set to these amounts, but the actual volume administered is unknown. It is highly likely that drug amounts greater than intended were inadvertently administered. Ivermectin may have been present on the outside of the pipette tip and come in contact with the oral mucosa, further increasing exposure to the drug. On the second day of administration, greater effort was made to clean the outside of the pipette to reduce ancillary exposure.

There was a change in ivermectin brand immediately before this treatment and it is possible that drug concentration in the bottle was significantly higher than in other formulations. This is unlikely as brands had been changed multiple times in the past without problem, the 1:10 dilution tested at 1.1 mg/ml concentration was from the same bottle, and after this event the bottle was used to treat other frogs following the SP without incident.

In many species, clinical signs begin to resolve within a few days although full recovery may take weeks (Hautekeete *et al*., 1998; Hopper *et al*., 2002; Kenny *et al*., 2008; Merola and Eubig, 2012; Plummer *et al*., 2006; Pritchard, 2010; Swor *et al*., 2009; Széll *et al*., 2001; Teare and Busch, 1983). In these frogs, clinical signs progressed over days 3 – 6. It is likely that supportive care was started too late and not optimal. Over the first week, frogs that had relatively low scores progressed to higher scores. It is possible that frogs developed secondary disease from effects of hypoxia, circulatory changes, electrolyte imbalance, negative energy balance, and/or bacterial infections rather than progression of toxicosis alone.

Additional supportive care is possible. Activated charcoal may reduce enterohepatic recirculation. Oxygen support would combat possible hypoxia from cardiovascular-respiratory compromise. Improved cardiac output and general stimulatory effects may have been achieved through caffeine via topical application of a weak tea solution and/or atropine administration (Wright, 2009; Wright and DeVoe, 2012). Other medications used periodically to manage ivermectin toxicosis include lipophilic intravenous emulsions, physiostigmine, flumazenil, and picrotoxin (Hopper *et al*., 2002; Roder and Stair, 1998; Trailović and Nedelijković, 2010) and

may have utility in amphibian cases. Heart rate was not monitored in these frogs but could be utilized to evaluate case status and response to treatment.

Dendrobates auratus and *D. tinctorius* azureus types had increased case presentation over *D. tinctorius* tinctorius types, where no toxicity was noted. It is likely that weight differences were largely responsible for this seeming group difference with lower weight animals receiving a higher dosage, however physiologic difference between species or types cannot be entirely excluded.

These animals were part of a collection of over 500 Dendrobatid and 200 other frog species being handled for a comprehensive chytrid fungus survey. In the months prior to this survey, the Dendrobatid group had experienced increased numbers of mortalities associated with intestinal nematodiasis. Husbandry and veterinary staff elected to opportunistically administer anti-helminthic medication and change cages at the time frogs were handled for the survey. More typically, anti-helminthic treatment was administered when clinical signs and diagnostic testing indicated nematode infection was contributing to poor health.

Frogs in this report appeared to have toxicosis from an overdose of ivermectin. The survival rate for frogs with clinical signs was 32% (10/31) and even animals with profound central nervous system depression recovered. Seventeen animals never developed clinical signs and the overall survival rate (animals that developed clinical signs and lived with animals that never showed clinical signs) was 56% (27/48). Improved supportive care might improve survival rates. Anecdotal reports of toxicosis exist but are not clearly represented in the literature. In the three

years subsequent to this event, ivermectin administration using the SP was continued without similar toxicity noted (Clayton, unpublished data). Accurate dosing with dilutions and micropipettes should be used in small frogs to reduce the chance of ivermectin toxicosis.

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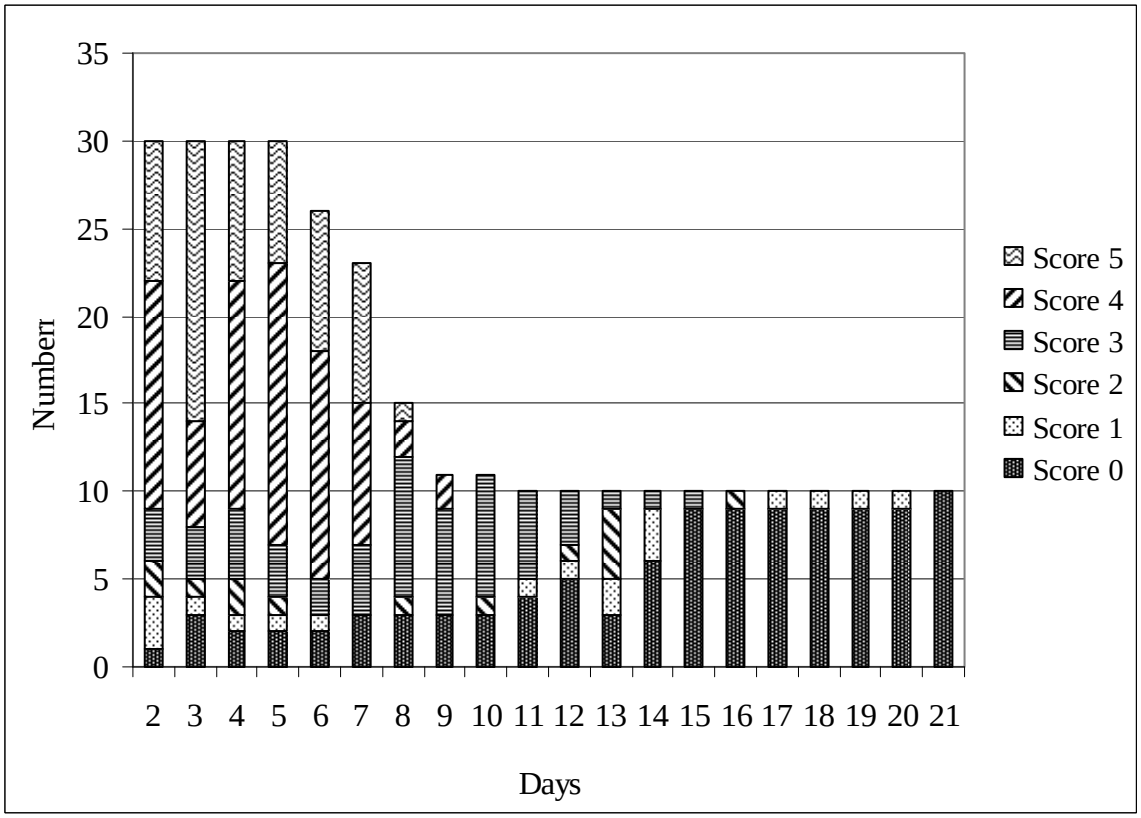
FIGURE 1. Scores over time in 30 dendrobatid frogs treated for presumptive ivermectin toxicity.*



* Excludes 1 of the 31 frogs that developed clinical signs and died on day 1.

TABLE 1. Species, sex, age, and weight on the day of treatment and outcome in dendrobatid frogs treated with ivermectin.

Animal	Species [a]	Sex [b]	Age (mo)	Weight (g)	Outcome [c]
1	auratus	U	18	3.03	D
2	auratus	U	14	2.73	D
3	auratus	U	15	2.53	D
4	auratus	F	15	4.48	S
5	auratus	F	65	4.44	D
6	auratus	F	65	4.66	S
7	auratus	F	65	3.98	D
8	auratus	U	15	2.46	D
9	auratus	U	22	2.54	D
10	auratus	U	18	2.88	S
11	auratus	M	14	2.82	S
12	auratus	U	13	2.25	D
13	auratus	U	18	3.10	D
14	auratus	U	18	2.78	D
15	auratus	U	19	2.83	D
16	auratus	M	14	2.88	S
17	auratus	M	65	3.16	S
18	auratus	U	14	3.54	D
19	auratus	U	21	2.30	D
20	azureus	F	14	4.66	S

21	azureus	F	14	3.60	D
22	azureus	M	14	5.06	D
23	azureus	M	38	4.16	D
24	azureus	F	49	5.13	D
25	azureus	M	22	4.00	D
26	azureus	F	35	4.51	S
27	azureus	F	21	4.59	S
28	azureus	M	22	3.55	D
29	azureus	F	19	5.14	D
30	azureus	M	19	4.82	S
31	azureus	F	13	5.32	S
32	azureus	M	114	4.10	D
33	tinctorius	M	127	6.20	S
34	tinctorius	F	91	6.64	S
35	tinctorius	M	170	6.58	S
36	tinctorius	F	124	8.13	S
37	tinctorius	M	16	4.37	S
38	tinctorius	F	15	7.84	S
39	tinctorius	F	91	7.67	S
40	tinctorius	M	19	5.76	S
41	tinctorius	M	19	4.97	S
42	tinctorius	F	10	4.54	S
43	tinctorius	M	20	5.90	S

44	tinctorius	F	11	4.99	S
45	tinctorius	M	140	5.23	S
46	tinctorius	F	153	9.12	S
47	tinctorius	F	91	7.61	S
48	tinctorius	M	116	6.03	S

[a] auratus = ***D. auratus***, azureus = ***D. tinctorious*** azureus type, tinctorius = ***D. tinctorius*** tinctorius type

[b] M = male, F = female, U = unknown

[c] D = death, S = survived

TABLE 2. Initial score and survival rate of 31 dendrobatid frogs with presumptive ivermectin toxicity.

Score	Clinical Signs	Number of Frogs	Survival Rate
0	Clinically normal	1	1/1 (100%)
1	Mild ataxia, normal spontaneous movement present	3	2/3 (67%)
2	Moderate ataxia, reduced spontaneous movement but reaction to light tactile stimuli	2	2/2 (100%)
3	Severe ataxia, reduced spontaneous movement but reaction to moderate tactile stimuli, righting reflex moderately impaired	3	1/3 (33%)
4	Maintains partially upright position, no spontaneous movement or movement in reaction to tactile stimuli, righting reflex significantly impaired	13	4/13 (31%)
5	Unable to maintain upright position, no reaction to noxious stimuli, complete flaccid paralysis, no righting reflex	9	0/9 (0%)

TABLE 3. Maximum score and survival rate of 31 dendrobatid frogs with presumptive ivermectin toxicity.

Score	Clinical Signs	Number of Frogs	Survival Rate
1	Mild ataxia, normal spontaneous movement present	3	3/3 (100%)
2	Moderate ataxia, reduced spontaneous movement but reaction to light tactile stimuli	0	-
3	Severe ataxia, reduced spontaneous movement but reaction to moderate tactile stimuli, righting reflex moderately impaired	0	-
4	Maintains partially upright position, no spontaneous movement or movement in reaction to tactile stimuli, righting reflex significantly impaired	8	4/8 (50%)
5	Unable to maintain upright position, no reaction to noxious stimuli, complete flaccid paralysis, no righting reflex	20	3/20 (15%)

TABLE 4. Logistic regression performed using weight, age, and sex as factors predicting mortality, a binary, categorical variable.

	B	S.E.	Wald	df	p-value	Exp(B)
Weight (g)	.962	.470	4.186	1	.041	2.618
Age (mo)	-.006	.012	.285	1	.593	.994
Sex	-.572	.889	.414	1	.520	.564
Constant	-2.524	2.034	1.540	1	.215	.080

Note: -2 Log likelihood = 36.113. R^2 = .164 (Cox & Snell), .236 (Nagelkerke).