Chapter 3 Amphibians

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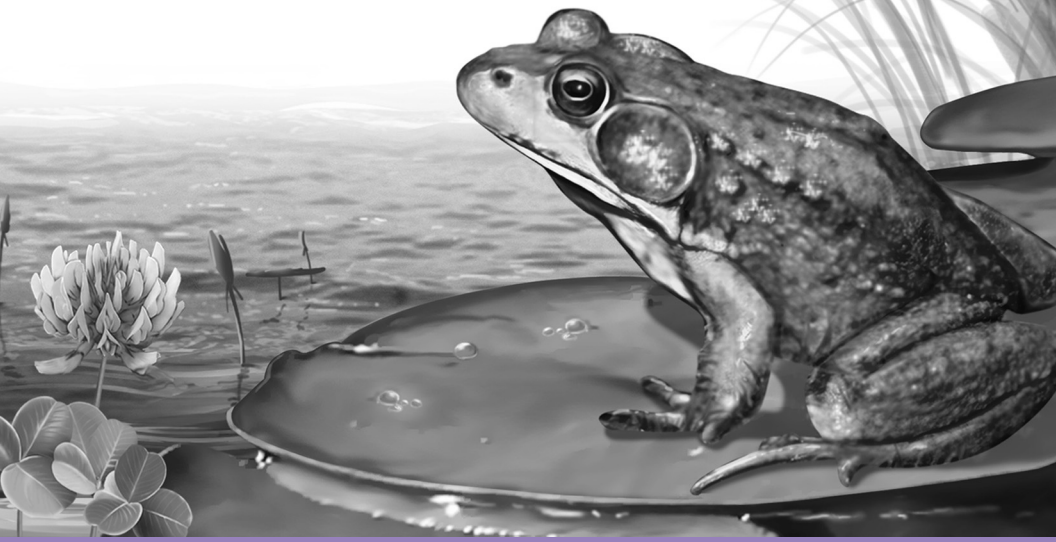

TABLE 3-1 Antimicrobial Agents Used in Amphibians.[a,b]

Agent	Dosage	Species/Comments
Amikacin	5 mg/kg IM q36h[59]	Bullfrogs/PK
	5-10 mg/kg SC, IM, ICe q24-48 h[58]	Most species; may be used in combination with piperacillin
Carbenicillin	100 mg/kg SC, IM q72h[59]	
	200 mg/kg SC, IM, ICe q24h[59]	
Ceftazidime	20 mg/kg SC, IM q48-72h[59]	
Chloramphenicol	50 mg/kg SC, IM, ICe q12-24h[59]	Caution: even miniscule exposure carries risk of aplastic anemia in susceptible individuals; wear disposable gloves when handling; aplastic anemia-like findings in *Bufo regularis* exposed to 125 mg/kg PO q24h × 12 wk[14]
	20 mg/L bath[a] changed daily[59]	
Ciprofloxacin	10 mg/kg PO,[59] ICe[58] q24h	
	500-750 mg/75 L as 6-8 hr bath[a] q24h[59]	May be used for large numbers of animals
Doxycycline (Psittavet, Vetafarm)	50 mg/kg IM q7d[59]	Broad-spectrum antibiotic, part of 4-quadrant therapy; may have antiinflammatory effect; chlamydiosis
Doxycycline (Vibramycin, Zoetis)	5-10 mg/kg PO q24h[59]	Chlamydiosis
	10-50 mg/kg PO q24h[59]	African clawed frogs/chlamydiosis
Doxycycline 1% topical gel, compounded	Apply topically q8-12h not to exceed 10 mg/kg per day[59]	Useful for localized lesions; may have antiinflammatory effect
Enrofloxacin	5-10 mg/kg PO, SC, IM q24h59	Most species/PK (bullfrogs);[59] ICe and topical routes also used but with limited PK data[59]
	10 mg/kg SC, IM[15]	African clawed frogs/PK; high kidney concentrations of enrofloxacin and ciprofloxacin;[15] no significant difference between routes[23]
	10 mg/kg topically[52]	Coqui frogs/detectable tissue concentration for >24 hr, no correlation to plasma concentration
	500 mg/L × 6-8 hr bath[a] q24h59	
Enrofloxacin and silver sulfadiazine solution (Baytril Otic, Bayer)	Apply topically to lesions q12h[59]	May have some antifungal effect, but does not appear effective against chytrid
Gentamicin	2-4 mg/kg IM q72h × 4 treatments[59]	
	2.5 mg/kg IM q72h[50]	Coldwater salamanders (i.e., *Necturus*)/PD; more frequent dosing may be needed if temperature >4°C (39.2°F)
	3 mg/kg IM q24h at 22.2°C (72°F)[59]	Leopard frogs/PD; at higher temperatures, serum concentrations will be lower

TABLE 3-1 Antimicrobial Agents Used in Amphibians. (cont'd)

Agent	Dosage	Species/Comments
Gentamicin (cont'd)	Topical to eyes[59]	All species/ocular infections; dilute to 2 mg/mL
	Intracameral injection once; not to exceed 4 mg/kg[59]	Panophthalmitis
Metronidazole	10 mg/kg PO q24h × 5-10 days[42]	For chronic diarrhea
	10 mg/kg IV q24h × 2 days[59]	Anaerobic infections
	12 mg/kg topically q24h × 5-10 days[59]	For chronic diarrhea
	20 mg/kg PO q48h × 20 days[59]	Anaerobic infections
	50 mg/kg PO q24h × 3 days[59]	Anaerobic infections
	60 mg/kg topically q24h × 3 days[59]	Anaerobic infections
	50 mg/L × 24 hr bath[a,59]	Anaerobic infections
Ofloxacin 0.3% ophthalmic solution	1 drop q2-4h × 10 days[58]	Keratitis; may also be applied topically to wounds
Oxytetracycline	25 mg/kg SC, IM q24h[59]	Most species
	50 mg/kg PO q12-24h[59]	Most species
	50-100 mg/kg IM q48h[59]	Bullfrogs/PK; especially useful in cases of chlamydiosis (use up to 30 days)[59]
	100 mg/L × 1hr bath[a,59]	Most species
	1 g/kg feed × 7 days[59]	Most useful with axolotls and *Xenopus* fed compounded pelleted diet[59]
Piperacillin	100 mg/kg SC, IM q24h[59]	Anaerobes; may be used in combination with amikacin
Silver sulfadiazine (Silvadine Cream 1%, Marion)	Topical q24h[59]	Antibiotic cream
Sulfadiazine	132 mg/kg PO q24h[59]	
Sulfamethazine	1 g/L bath[a] to effect[59]	Change daily
Tetracycline	50 mg/kg PO q12h[59]	
	150 mg/kg PO q24h × 5-7 days[59]	
	167 mg/kg (5 mg/30 g) PO q12h × 7 days[59]	
Trimethoprim/sulfa	3 mg/kg PO, SC, IM q24h[59]	Unspecified sulfa
Trimethoprim/ sulfadiazine	15-20 mg/kg IM q48h[59]	Chronic diarrhea[59]
Trimethoprim/ sulfamethoxazole	15 mg/kg PO q24h[59]	Chronic diarrhea

[a]Water baths containing antibiotics or topical applications may not provide as consistent distribution as parenteral administration.
[b]SC can be administered in the dorsal lymph sac of anurans.[59]

TABLE 3-2 Antifungal Agents Used in Amphibians.

Agent	Dosage	Species/Comments
Amphotericin B	1 mg/kg ICe q24h[59]	Internal mycoses; acutely toxic to *Alytes muletensis* tadpoles at 8 μg/mL bath[33]
Benzalkonium chloride	0.25 mg/L × 72 hr bath[59]	
	2 mg/L × 1 hr bath q24h[59]	Saprolegniasis
Chloramphenicol	20 mg/kg topically (applied as Chlorsig 1% ointment [Sigma] which also contains paraffin and wool fat)[5]	Chytridiomycosis; safe for larvae, recent metamorphs, and adults; confirm negative result by real-time PCR;[5,59] caution: even miniscule exposure carries risk of aplastic anemia in susceptible individuals; wear disposable gloves when handling; aplastic anemia-like findings in *Bufo regularis* exposed to 125 mg/kg PO q24h × 12 wk[14]
	10-30 mg/L (10-30 ppm) as continuous bath replaced fresh daily for up to 30 days[59]	
	20 mg/L by continuous shallow immersion × 14 days, changed daily[61]	Australian green frog (*Litoria caerulea*); severely ill frogs treated with combination of chloramphenicol, SC fluids q8-12h × 6 days, and temperature increased to 28°C × 14 days[61]
Florfenicol	10 μg/mL topical spray q24h × 14 days[39]	Experimentally infected *Alytes muletensis* adults/reduced zoosporangia numbers but did not eliminate infection; GI and renal toxicity to tadpoles at 100 μg/mL[39]
	30 ppm as continuous bath replaced fresh daily for up to 30 days[59]	Chytridiomycosis; safe for larvae, recent metamorphs, and adults; confirm negative result by real-time PCR[59]
Fluconazole	60 mg/kg PO q24h[59]	
Itraconazole	10 mg/kg PO q24h[59]	Topical route best choice to treat chytridiomycosis; caution with tadpoles[17,59]
	0.01% in 0.6% salt solution × 5 min bath q24h × 11 days[59]	
	0.01% in buffered solution × 5 min bath q24h × 11-14 days[18]	Multiple species/cleared chytridiomycosis by PCR 14 days post-treatment; 6-15 mo post-treatment follow-up yielded positive PCR in some individuals
	0.5-1.5 mg/L × 5 min bath q24h × 7 days[17]	*Alytes muletensis* tadpoles/safe at varying concentrations and duration of 7-28 days; confirmed negative PCR post-treatment; varying levels of depigmentation observed in all individuals[17]
	50 mg/L × 5 min bath q24h × 10 days[25]	Multiple species/cleared chytridiomycosis in subclinical animals; confirmed with PCR

TABLE 3-2 Antifungal Agents Used in Amphibians. (cont'd)

Agent	Dosage	Species/Comments
Itraconazole (cont'd)	0.0025% × 5 min bath q24h × 6 days[8]	Australian green tree frog (*Litoria caerulea*), coastal plains toad (*Incillus nebulifer*)/cleared PCR positive juveniles with no clinically apparent side effects
Ketoconazole	10-20 mg/kg PO q24h[59] Topical cream[59]	
Methylene blue	2-4 mg/L bath to effect[59] 4 mg/L × 1 hr bath q24h[59]	Tadpoles/may reduce mortality in newly hatched tadpoles Saprolegniasis
Miconazole	5 mg/kg ICe q24h × 14-28 days[59] Topical cream or solution[59]	Systemic mycoses Topical route best choice for chytridiomycosis; solutions containing alcohol may cause irritation; do not use with larvae[59]
Neomycin, polymixin B, bacitracin (Neosporin, Pfizer)	Apply topically to wound q24h[20]	Microsporidian infections; not recommended for bacterial infections, appears to inhibit re-epithelialization[59]
Nystatin 1% cream	Topical[59]	Cutaneous mycoses
Potassium permanganate	1:5000 water × 5 min bath q24h[59]	Cutaneous mycoses
Sodium chlorite ($NaOCl_2$)	20 mg/L × 6-8 hr bath[59]	Cutaneous mycoses
Temperature elevation	30°C (86°F) × 10 days[11] 37°C (98.6°F) for 16 hr[56]	*Rana catesbeiana, Acris crepitans*/confirm negative result by real-time PCR[11] Chytridiomycosis, caution with temperature elevation in sensitive species
Terbinafine hydrochloride (Lamisil AT, Novartis)	0.005%-0.01% in distilled water × 5 min bath q24h × 5 days, or q48h × 6 treatments[7]	Various species/no adverse clinical effects noted with treatment; pH 7.0; confirm negative result by real-time PCR[7]
Voriconazole	1.25 μg/mL q24h topically via spray × 7 days[33]	Poison dart frogs, Iberian midwife toad (*Alytes cisternasii*)/cleared chytridiomycosis in naturally infected individuals in vivo; performed poorly with in vitro assays[33]
Voriconazole (V) + polymixin E (P) + elevated temperature (T)	(V) 12.5 μg/mL q24h topically via spray + (P) 2000 IU/mL × 10 min bath q12h + (T) 20°C (68°F) continuous × 10 days[6]	Fire salamanders/treatment of *Batrachochytrium salamandrivorans*; no effect of medications at 15°C (59°F)[6]

TABLE 3-3 Antiparasitic Agents Used in Amphibians.[a]

Agent	Dosage	Species/Comments
Acriflavin	0.025% bath × 5 days[59]	Protozoa
	500 mg/L × 30 min bath[59]	Protozoa
Benzalkonium chloride	2 mg/L × 1 hr bath q24h to effect[59]	Protozoa
Distilled water	3 hr bath[59]	Protozoa
Febantel (in combination with pyrantel pamoate and praziquantel; Drontal Plus, Bayer)	0.01 mL/1 g (10 mL/kg) PO q2-3wk[40]	Nematodes, cestodes, possibly trematodes
Fenbendazole	—	Fenbendazole combinations follow
	30-50 mg/kg PO[59]	Gastrointestinal nematodes
	50 mg/kg PO q24h × 3-5 days, repeat in 14-21 days[59]	Gastrointestinal nematodes
	50-100 mg/kg PO[42] repeat in 2-3 wk prn	Most species/gastrointestinal nematodes
	100 mg/kg PO,[59] repeat in 14 days	Gastrointestinal nematodes
Fenbendazole (F)/ ivermectin (I)	(F) 100 mg/kg PO on day 1, then (I) 0.2 mg/kg PO on days 2,11[59]	Gastrointestinal nematodes
Fenbendazole (F)/ metronidazole (M)	(F) 100 mg/kg PO, repeat in 10-14 days + (M) 10 mg/kg PO q24h for 5 days[59]	Concurrent gastrointestinal nematodes and protozoa
Formalin (10%)	—	Do not use if skin is ulcerated; may be toxic to some species
	1.5 mL/L × 10 min bath q48h to effect[59]	Protozoans; may be toxic in some species
	0.5% × 10 min bath once[59]	Monogenic trematodes; may be toxic to some species
Ivermectin	—	See fenbendazole for combination; caution: may cause flaccid paralysis with overdosage; caffeine or physostigmine may ameliorate effects[59]
	0.2-0.4 mg/kg PO, SC, repeat q14d as needed[59]	Nematodes, including lungworms; mites
	2 mg/kg topically, repeat in 2-3 wk[30]	Especially useful for small specimens[59] and *Rana* spp.[30]
	10 mg/L × 60 min bath, repeat q14d prn[59]	Mites
Levamisole	—	May cause paralysis in some species at suggested dosages;[59] caffeine or physostigmine may ameliorate effects[59]
	6.5-13.5 mg/kg topically to pelvic patch, repeat in 10 days[4]	*Anaxyrus houstonensis*/reduced nematode egg counts
	10 mg/kg IM, ICe, topically,[59] repeat in 2 wk	Nematodes, including lungworms
	12 mg/L bath × 4 days[24]	African clawed frogs/cutaneous nematodes; use ≥4.2 L of tank water/frog

TABLE 3-3 Antiparasitic Agents Used in Amphibians. (cont'd)

Agent	Dosage	Species/Comments
Levamisole (cont'd)	100 mg/L × ≥72 hr bath[59]	Resistant nematodes
	100-300 mg/L × 24 hr bath, repeat in 1-2 wk[59]	Nematodes, including subcutaneous nematodes in aquatic amphibians; water soluble form is available through aquaculture supply companies
Metronidazole	—	See fenbendazole for combination; toxicity possible at high doses
	10 mg/kg PO q24h × 5-10 days[59]	Protozoa; for unfamiliar or sensitive species
	50 mg/kg PO q24h × 3-5 days[59]	Confirmed cases of amoebiasis and flagellate overload
	100 mg/kg PO q3d[59]	Protozoa
	100-150 mg/kg PO, repeat in 2-3 wk or prn[59]	Protozoa (i.e., *Entamoeba, Hexamita, Opalina*)
	50 mg/L × 24 hr bath[59]	Aquatic amphibians/protozoa
	500 mg/100 g feed × 3-4 treatments[59]	Ciliates
Moxidectin	200 μg/kg SC q4mo[44]	Nematodes
Oxfendazole	5 mg/kg PO[59]	Gastrointestinal nematodes
Oxytetracycline	25 mg/kg SC, IM q24h[59]	Protozoa
	50 mg/kg PO q12h[59]	Protozoa
	1 g/kg feed × 7 days[59]	Protozoa
Paromomycin	50-75 mg/kg PO q24h[59]	Gastrointestinal protozoa
Piperazine	50 mg/kg PO, repeat in 2 wk[59]	Gastrointestinal nematodes
Ponazuril	30 mg/kg PO q12h × 3 days, repeat in 3 wk; often more effective at 30 mg/kg PO q24h × 30 days; may work with less frequent treatments[59]	Coccidia but not *Cryptosporidium*; may have some effect on unidentified protozoan cysts
Potassium permanganate	7 mg/L × 5 min bath q24h to effect[59]	Ectoparasitic protozoa
Praziquantel	8-24 mg/kg PO, SC, ICe, topically,[59] repeat q14d	Trematodes, cestodes
	10 mg/L × 3 hr bath,[59] repeat q7-21d	Trematodes, cestodes
Pyrantel pamoate	5 mg/kg PO q14d[40]	Nematodes
Ronidazole	10 mg/kg PO q24h × 10 days[59]	Flagellated protozoa, amoebas
Salt (sodium chloride)	4-6 g/L continuous bath[59]	Ectoparasitic protozoa
	5 g/L bath up to 12h, 10 g/L bath up to 1h[32]	Axolotls, immediate negative clinical effects in baths >20 g/L[32]
	6 g/L × 5-10 min bath q24h × 3-5 days[59]	Ectoparasitic protozoa
	25 g/L × ≤10 min bath[59]	Ectoparasitic protozoa
Selamectin (Revolution, Zoetis)	6 mg/kg topically[12]	Bullfrogs/PK

Continued

TABLE 3-3 Antiparasitic Agents Used in Amphibians. (cont'd)

Agent	Dosage	Species/Comments
Sulfadiazine	132 mg/kg PO q24h[59]	Coccidiosis
Sulfamethazine	1 g/L bath[59]	Coccidiosis; change daily to effect
Tetracycline	50 mg/kg PO q12h[59]	Protozoa
Thiabendazole	50-100 mg/kg PO,[59] repeat in 2 wk prn	Gastrointestinal nematodes
	100 mg/L bath, repeat in 2 wk[59]	Verminous dermatitis
Trimethoprim/sulfa	3 mg/kg PO, SC, IM q24h[59]	Coccidiosis; unspecified sulfa

[a]SC can be administered in the dorsal lymph sac of anurans.[59]

TABLE 3-4 Chemical Restraint/Anesthetic/Analgesic Agents Used in Amphibians.[a]

Agent	Dosage	Species/Comments
Alfaxalone	5-25 mg/kg IM[28]	Most species/recommend starting at lower dose (5-10 mg/kg) and titrating up
	10-17.5 mg/kg IM[41]	Bullfrogs/immobilization, respiratory depression, still responsive to noxious stimuli; dose dependent time to recumbency and time to recovery; no effect by immersion at 2 g/L for 30 min[41]
	18 mg/kg IM, IV, ICe[22]	African clawed frogs/deep sedation for 1-3 hr (IM, IV), 10-60 min ICe; no effect via immersion at 18 mg/L[22]
	20-30 mg/kg IM[46]	Australian tree frogs/initial effect within 10 min, respiratory depression; insufficient anesthesia as sole agent for painful procedures
	5 mg/L in fresh water bath[35]	Axolotls/single individual; induction of anesthesia, maintained continuous irrigation of gills and skin with additional 0.03 mL drops of alfaxalone for maintenance of anesthesia during surgery[35]
	200 mg/L in fresh water bath[2]	Fire-bellied toads/buffer with sodium bicarbonate to pH 7.2; anesthetic induction in 14 ± 4 min, variable duration of anesthesia up to 30 min; not sufficient for painful procedures
Alfaxalone (A)/ morphine (M)	(A) 3 mg/100 mL + (M) 5 mg/100 mL as bath[1]	Fire-bellied toads/provided anesthetic induction and antinociception
Atipamezole (Antisedan, Zoetis)	Titrate to effect IM, IV	Antagonist for dexmedetomidine[31]

TABLE 3-4 Chemical Restraint/Anesthetic/Analgesic Agents Used in Amphibians. (cont'd)

Agent	Dosage	Species/Comments
Benzocaine (Sigma Chemical)	—	Anesthesia; not sold as fish anesthetic in United States; available from chemical supply companies; do not use topical anesthetic products marketed for mammals; prepare stock solution in ethanol (poorly soluble in water); store in dark bottle at room temperature
	50 mg/L bath to effect[59]	Larvae/dissolve in ethanol first
	200-300 mg/L bath to effect[59]	Frogs, salamanders/dissolve in ethanol first
	200-500 mg/L bath[59]	Dissolve in acetone first
Buprenorphine	38 mg/kg SC[31]	Analgesia >4 hr; ED_{50}[b] in leopard frogs[31]
	50 mg/kg ICe q24h[26]	Eastern red spotted newts/return to normal behavior following limb amputation; may take >1 hr for onset of clinical effects; postsurgical bath in 0.1% sulfamerazine (w/v; Sigma Chemical Company)[26]
Butorphanol	0.2-0.4 mg/kg IM[59]	Analgesia; efficacy uncertain[59]
	0.5 mg/L continuous immersion for 3 days[26]	Eastern red spotted newts/return to normal behavior following limb amputation; may take >4 hr for onset of clinical effects; postsurgical bath in 0.1% sulfamerazine (w/v; Sigma Chemical Company)[26]
Clove oil (eugenol)	0.3 mL/L (~310-318 mg/L)[59]	Anesthesia; deep anesthesia after 15 min bath; caused reversible gastric prolapse in 50% of leopard frogs
	0.35 mL in 1 L purified water[19]	African clawed frogs/anesthetic plane for frogs <10 g after 5 min immersion, for frogs ~30 g after 10 min immersion
	0.45 mL/L (~473 mg/L)[38]	Anesthesia; deep anesthesia induced in 80% of tiger salamanders
Codeine	53 mg/kg SC[31]	Analgesia >4 hr; ED_{50}[b] in leopard frogs
Dexmedetomidine	40-120 mg/kg SC[31]	Analgesia >4 hr; ED_{50}[b] in leopard frogs
Diazepam	—	See ketamine for combination
Fentanyl	0.5 mg/kg SC[31]	Analgesia >4 hr; ED_{50}[b] in leopard frogs
Flunixin meglumine	25 mg/kg intralymphatic[10]	African clawed frogs
Isoeugenol (Aqui-S; 0.54 μg/mL isoeugenol)	20-50 μL/L[47]	*Litoria ewingii* tadpoles/higher doses resulted in faster induction and longer recovery

Continued

TABLE 3-4 Chemical Restraint/Anesthetic/Analgesic Agents Used in Amphibians. (cont'd)

Agent	Dosage	Species/Comments
Isoflurane	—	Anesthesia; induction chamber
	3%-5% induction, 1%-2% maintenance[59]	Terrestrial species
	5%[59]	Terrestrial species/euthanasia; induction chamber
	Topical application of liquid isoflurane[59]	*Bufo* spp. (0.015 mL/g BW), African clawed frogs (0.007 mL/g BW)/ induce in closed container; once induced, remove excess from animal
	Topical mixture of isoflurane (3 mL), KY jelly (3.5 mL), and water (1.5 mL) [59]	*Bufo* spp. (0.035 mL/g BW), African clawed frogs (0.025 mL/g BW)/ induce in closed container; once induced, remove excess from animal
	Topical mixture of 1.5 parts distilled water, 3.5 parts nonspermicidal jelly, and 1.8 parts isoflurane[63]	American tree frogs/induced in closed container; once induced remove excess from animal; erythematous lesions and signs of systemic illness noted following application[63]
	0.28 mL/100 mL bath[59]	Induce in closed container
	Bubbled into water to effect[59]	Aquatic species
Ketamine	—	May have long induction and recovery times; does not provide good analgesia so may not be suited for major surgical procedures; other agents preferred; ketamine combination follows; see lidocaine
	50-150 mg/kg SC, IM[59]	Most species
Ketamine (K)/ diazepam (D)	(K) 20-40 mg/kg + (D) 0.2-0.4 mg/kg IM[59]	Variable results
Lidocaine 1%-2%	Local infiltration[59]	All/local anesthesia; with or without epinephrine; 2% lidocaine in combination with ketamine has been used for minor surgeries;[59] use with caution
Meloxicam	0.1 mg/kg[37]	American bullfrogs/decreased circulating prostaglandin E2 (PGE2) levels measured 24 hr post muscle biopsy[37]
	0.4-1 mg/kg PO, SC, ICe q24h[59]	Analgesia
Metomidate hydrochloride	30 mg/L bath[13]	*Rana pipiens*/immersion for 60 min then transferred to amphibian Ringer's solution; clinical sedation in 11/11 frogs; surgical anesthesia in 3/11; prolonged recovery; not recommended as sole anesthetic agent
Morphine	38-42 mg/kg SC[31]	Analgesia >4 hr
Nalorphine	122 mg/kg SC[31]	Analgesia >4 hr

TABLE 3-4 Chemical Restraint/Anesthetic/Analgesic Agents Used in Amphibians. (cont'd)

Agent	Dosage	Species/Comments
Naloxone	10 mg/kg SC;[31] titrate to effect	Antagonist for buprenorphine, butorphanol, codeine, fentanyl, morphine
Naltrexone	1 mg/kg SC;[31] titrate to effect	Antagonist for buprenorphine, butorphanol, codeine, fentanyl, morphine
Pentobarbital sodium	60 mg/kg IV, ICe[59]	Euthanasia; can also be administered in lymph sacs in anurans
Pentobarbital sodium + sodium phenytoin	1100 mg/kg + 141 mg/kg ICe[51]	African clawed frogs/complete cardiac arrest within 3 hr
Propofol	10-30 mg/kg ICe[59]	White's tree frogs/pilot study; use the lower dosage for sedation or light anesthesia; induction within 30 min; recovery in 24 hr
	35 mg/kg ICe[38]	Deep anesthesia in 83% of tiger salamanders[38]
	35 mg/kg ICe[57]	Sonoran desert toads/sedation only; did not achieve surgical plane of anesthesia
	60-100 mg/kg ICe[59]	Euthanasia
	88 mg/L by immersion[21]	African clawed frogs/induced for 15 min, then rinsed; respiratory depression, darkened skin color; death at doses over 175 mg/L
	100-140 mg/kg topically[59]	Maroon-eyed tree frogs (*Agalychnis litodryas*)/unpublished data; 15-20 min to max effect at 100 mg/kg dose; 10-15 min to max effect at 140 mg/kg;[59] sedation to deep anesthesia; remove and rinse when desired level achieved; recommended only for animals <50 g
Sevoflurane	Topical application	Rapid recovery unless constant reapplication
	Topical mixture of 1.5 parts distilled water, 3.5 parts nonspermicidal jelly, and 3 parts sevoflurane[48,63]	American tree frogs/induced in closed container with 2 mL of sevoflurane jelly per individual; once induced, remove excess from animal; recovery 4.5 times faster than topical isoflurane jelly;[63] in cane toads, reliable loss of righting reflex when 37.5 μg/g sevoflurane in jelly was applied to dorsum[48]
Tiletamine/ zolazepam (Telazol, Fort Dodge)	10-20 mg/kg IM[59]	Results variable between species; rapid recovery; not suitable as single anesthetic agent for anurans[29]

Continued

TABLE 3-4 Chemical Restraint/Anesthetic/Analgesic Agents Used in Amphibians. (cont'd)

Agent	Dosage	Species/Comments
Tricaine methanesulfonate (MS-222) (Finquel, Argent)	—	Anesthesia; buffer the acidity by adding sodium bicarbonate to buffer the solution to a pH of 7.0-7.1; aerate water to prevent hypoxemia; remove from bath on induction or overdosing can readily occur; following bath, place terrestrial amphibians on moist towel or in very shallow water to recovery; some species can be induced at much lower concentrations than listed here; in some cases, anesthesia can be maintained by dripping a dilute solution of this drug (100-200 mg/L) over the skin or by covering animal with a paper towel moistened with the anesthetic[59]
	50-200 mg/kg SC, IM, ICe[59]	Most species/may be irritating administered SC, IM (neutral solution is preferred)[59]
	100-200 mg/kg ICe[49]	Leopard frogs
	100-400 mg/kg ICe[49]	Bullfrogs
	100-200 mg/L bath to effect[59]	Larvae/induction
	200-500 mg/L bath to effect[59]	Tadpoles, newts/induction in 15-30 min
	0.5-2 g/L bath to effect[59]	Frogs, salamanders/induction in 15-30 min
	1 g/L bath to effect[59]	Most gill-less adult species (unless very large)/induction
	1 g/L by immersion, buffered with 1 g/L sodium bicarbonate[57]	Sonoran desert toads/surgical plane of anesthesia
	1-2 g/L by immersion[27]	African clawed frogs/buffered to pH of 7.0 ± 0.4; 20 min induction then rinsed; respiratory depression; longer duration of surgical anesthesia with higher dosing
	2-3 g/L bath to effect[59]	Toads/induction in 15-30 min
	5 g/L immersion[51]	African clawed frogs/immersion for 1 hr; death within 3 hr
	10 g/L bath[59]	Euthanasia; can be administered ICe or in lymph sacs

[a]SC can be administered in dorsal lymph sac in anurans.[59]
[b]ED_{50}, effective dose for 50% of the population.

TABLE 3-5 Hormones Used in Amphibians.[a]

Agent	Dosage	Species/Comments
Gonadotropin-releasing hormone (GnRH)	10 μg SC to female followed by additional 20 μg after 18 hr; 5 μg SC to male[54]	Tomato frogs (*Dyscophus guineti*)/ovulation and spermiation
	0.1 mg/kg SC, IM, repeat prn[59]	Induction of ovulation in those non-responsive to pregnant mare serum gonadotropin (PMSG) or human chorionic gonadotropin (hCG); administer to females 8-12 hr before males
Human chorionic gonadotropin (hCG)	50-300 U[59] SC, IM	For mating or release of sperm in males; follow with GnRH in 8-24 hr
	250-400 U SC, IM[59]	African clawed frogs, axolotls/induction of ovulation; may be used with PMSG and/or progesterone
Luteinizing hormone–releasing hormone (LHRH)	5 μg ICe per animal[53]	Salamanders (*Desmognathus ochrophaeus*)/induced oviposition in 94% of animals
	10 μg in 0.05 mL of 40% DMSO applied to ventral drink patch[43]	*Bufo americanus*, *B. valliceps*/induced spermiation in 70% of males
Pregnant mare serum gonadotropin (PMSG)	50-200 U SC, IM[59]	African clawed frogs, axolotls/induction of ovulation; administer 600 U hCG SC, IM 72 hr later[59]
Progesterone	1-5 mg SC, IM[59]	African clawed frogs, axolotls/use in addition to PMSG or hCG for induction of ovulation

[a]SC can be administered into the dorsal lymph sac of anurans.[59]

TABLE 3-6 Miscellaneous Agents Used in Amphibians.[a]

Agent	Dosage	Species/Comments
Amphibian Ringer's solution (ARS)	6.6 g NaCl, 0.15 g KCl, 0.15 g $CaCl_2$, and 0.2 g $NaHCO_3$ in 1 L water[59]	For treating hydrocoelom and subcutaneous edema; place animal in shallow ARS bath until stabilized (≈24 hr or more); replace with fresh solution daily; may need to wean animal off ARS by placing it in gradually more dilute solutions; hypertonic solution created by using 800-950 mL water instead of 1 L and may be more effective for some cases of hydrocoelom; up to 10 g of glucose may be added per L, but then solution must be made fresh daily[59]
Atropine	0.1 mg/animal SC, IM prn[59]	Organophosphate toxicosis
Caffeine	Use caffeinated tea bag; steep (soak) until solution is "weak tea"; place amphibian in shallow bath, replace q6h[59]	Stimulant; may help reverse ivermectin or levamisole toxicosis, or excessively deep anesthesia[59]
Calcium glubionate (Calcionate, 1.8 g/5 mL, Rugby Laboratories)	1 mL/kg PO q24h[59]	Nutritional secondary hyperparathyroidism

Continued

TABLE 3-6 Miscellaneous Agents Used in Amphibians. (cont'd)

Agent	Dosage	Species/Comments
Calcium gluconate	100-200 mg/kg SC[59]	Hypocalcemic tetany
	2.3% continuous bath (with 2-3 U/mL vitamin D_3)[59]	Nutritional secondary hyperparathyroidism
Critical care diets	—	Dosages are approximate; may be more appropriate to offer larger volume less frequently for easily stressed animals
• Carnivore Critical Care (Oxbow)	3% bodyweight PO q24-72h[59]	
• Emeraid for Carnivores (Lafeber)	3% body weight PO q24-48h[59]	
• Feline Clinical Care Liquid (Pet-Ag)	1-2 mL/50 g PO q24h[59] 3-6 mL/50 g PO q72h[59]	
• Hill's Feline a/d (Hill's Pet Nutrition)	PO[59]	Nutritional support; mix 1:1 with water; generally gavaged
Cyanoacrylate surgical adhesive (Vet Bond, 3M)	Topical on wounds[59]	Produces a seal for aquatic and semiaquatic species
Dexamethasone	1.5 mg/kg SC, IM[59]	Vascularizing keratitis
	1.5 mg/kg IM, IV[59]	Shock
Dextrose 5% solution	Bath[59]	For treating hydrocoelom and subcutaneous edema;[59] place animal in shallow bath until stabilized (≈24 hr or more); replace with fresh solution daily; may need to wean animal off dextrose by placing it in gradually more dilute solutions; 7.5%-10% solutions may be more effective for some cases of hydrocoelom
	Topically to affected tissues[34]	Small amount can be applied to edematous/inflamed tissue in cases of cloacal prolapse to aid in prolapse reduction
Doxycycline	1.25-2.5 mg/kg PO, SC, ICe q24h[59]	Antiinflammatory
	1% gel topically q12h[59]	Antiinflammatory
Hetastarch (6% in 0.9% saline)	Bath not to exceed 1 hr without reassessment[59]	May help with initial treatment of hydrocoelom
Hypertonic saline, 5% ophthalmic solution	Topically to affected tissues[34]	Small amount can be applied to edematous/inflamed tissue in cases of cloacal prolapse to aid in prolapse reduction
Laxative (Laxatone, Evsco)	PO[59]	Laxative, especially for intestinal foreign bodies
Meloxicam	0.4-1 mg/kg PO, SC, ICe q24h[58]	Antiinflammatory; presumptive analgesia; adjunct therapy for septicemia
	0.5% gel topically q24h; do not exceed 0.4 mg/kg[59]	Antiinflammatory for localized wounds
Methylene blue	2 mg/mL bath to effect[59]	Nitrite and nitrate toxicoses

TABLE 3-6 Miscellaneous Agents Used in Amphibians. (cont'd)

Agent	Dosage	Species/Comments
Oxygen	100% for up to 24 hr[59]	Adjunct treatment for septicemia, toxicoses
Physostigmine (ophthalmic drops)	1 drop/50 g topically q1-2h to effect[59]	May ameliorate flaccid paralysis from ivermectin or levamisole toxicosis
Prednisolone sodium succinate	5-10 mg/kg IM, IV[59]	Shock
Sodium thiosulfate	1% solution as continuous bath to effect[59]	Halogen toxicoses
Vitamin A (Aquasol A, 50,000 U/mL, Mayne Pharma)	Dilute 1:9 with sterile water; make fresh weekly; apply 1 drop from a tuberculin syringe with 27 g needle to amphibians under 5 g; 1 drop from tuberculin syringe w/out needle is about 200 U and useful for 15-30 g BW; >30 g, try 1 drop per 10 g BW; topically q24h × 14 days, then q4-7d[59]	Hypovitaminosis A; given the plethora of organ systems that hypovitaminosis A may affect, it is reasonable to institute vitamin A supplementation of any clinically ill amphibian, particularly ones with signs similar to "short tongue syndrome," swollen eyelids, evidence of infectious dermatitis, hydrocoelom, or simply "failing to thrive";[59] the use of mixed dietary carotenoids may also be effective in some species[9]
	Dilute 1:10 in sterile water; applied as one drop from18g needle; estimated as 50 U/frog q48h to q7d[45]	African foam-nesting frogs/weight range, 2-7 g; dosing q48h and once weekly significantly increased whole body vitamin A levels over control group and group treated with vitamin A fortified supplement dusted over crickets[45]
	1 U/g PO daily × 14 days[59]	
Vitamin A gel caps (10,000 U/cap)	Dilute 1:9 with corn oil to yield 1000 U/mL; give 1 U/g PO q24h × 14 days, then q7d[59]	Hypovitaminosis A; given the plethora of organ systems that hypovitaminosis A may affect, it is reasonable to institute vitamin A supplementation of any clinically ill amphibian, particularly ones with signs similar to "short tongue syndrome," swollen eyelids, evidence of infectious dermatitis, hydrocoelom, or simply "failing to thrive;"[59] the use of mixed dietary carotenoids may also be effective in some species[9]
Vitamin B_1	25 mg/kg PO[59]	Deficiency resulting from thiaminase-containing fish
	25-100 mg/kg IM, ICe[60]	
Vitamin D_3	2-3 U/mL continuous bath (with 2.3% calcium gluconate) [59]	Nutritional secondary hyperparathyroidism
	100-400 U/kg PO q24h[59]	
Vitamin E (alpha-tocopherol)	1 mg/kg PO, IM q7d[59]	
	200 U/kg feed[59]	Steatitis

[a]SC can be administered into the dorsal lymph sac of anurans.[59]

TABLE 3-7 Physiologic and Hematologic Values of Select Amphibians.[59]

Measurement	African clawed frog (*Xenopus laevis*)[5559]	American bullfrog (*Rana catesbeiana*)[59]	Australian common green tree frog (*Litoria caerulea*) [62]	Australian white-lipped tree frog (*Litoria* infrafrenata)[62]	Cuban tree frog (*Hyla septentrionalis*)[59]	Leopard frog (*Rana pipiens*)[59] ♂	Leopard frog (*Rana pipiens*)[59] ♀	Tiger salamander (*Ambystoma tigrinum*)[59]
BW (g)	—	—	—	—	28-35	25-42	25-46	35
Blood volume (mL/100 g BW)	—	3.1-3.6	—	—	7.2-7.8	—	—	—
Hematology[a]								
PCV (%)	23.3-47.0	39-42	34-40.8	26.0-34.0	20-24	19-52	16-51	40
RBC ($10^6/\mu L$)	0.80-1.48	0.45	0.62-0.82	0.63-0.82	—	0.23-0.77	0.17-0.70	1.66
Hgb (g/dL)	6.06-15.19	9.3-9.7	8.0-10.6	6.1-8.2	5.6-6.8	3.8-14.6	2.7-14	9.4
MCV (fL)	31.6-62.8	—	461-602	374-486	—	722-916	730-916	—
MCH (pg)	6.9-22.1	—	111-148	84-115	—	182-221	182-238	—
MCHC (g/dL)	19.3-32.3	21.1-25.9	236-268	210-250	25-31	22.7-26.8	19.9-27.7	—
WBC[b] ($10^3/\mu L$)	0.64-9.56	—	12.4-22.1	14.2-29.1	—	3.1-22.2	2.8-25.9	4.6
Early stages[b] (%)	—	—	—	—	—	—	—	—
Neutrophils[b] (%)	8 ± 1.1	—	14-27	15.0-32.0	—	—	—	—
Lymphocytes[b] (%)	65.3 ± 2.7	—	—	57.0-78.3	—	—	—	—
Monocytes[b] (%)	0.5	—	5.0-10.0	4.0-8.0	—	—	—	—
Eosinophils[b] (%)	—	—	1.0-5.0	0-1.3	—	—	—	—
Basophils[b] (%)	8.5 ± 1.4	—	0	0-1.0	—	—	—	—
Plasmocytes[b] (%)	0.2	—	—	—	—	—	—	—
Thrombocytes ($10^3/\mu L$)	17.1	—	23.2-33.5	25.8-38.8	—	—	—	—

Chemistry

ALP (U/L)	59-282	—	—	—	—	—	—	—
ALT (U/L)	10-39	—	—	—	—	—	—	—
AST (U/L)	27-1774	—	66-122	41-119	—	—	—	—
Bilirubin, total (mg/dL)	0.01-0.26	—	—	—	—	—	—	—
BUN (mg/dL)	2-10	—	—	—	—	—	—	—
Calcium (mg/dL)	5.2-12.3	—	10.6-13.1	8.6-11.3	—	—	—	—
Chloride (mEq/L)	72.7-92.7	—	—	—	—	—	—	—
Cholesterol (mg/dL)	56-563	—	—	—	—	—	—	—
Creatine kinase (U/L)	10-5400	—	347-705	233-722	—	—	—	—
Creatinine (mg/dL)	0.1-1.1	—	—	—	—	—	—	—
GGT (U/L)	1-19	—	—	—	—	—	—	—
Glucose (mg/dL)	18-111	—	55-78	45-81	—	—	—	—
LDH (U/L)	21-240	—	—	—	—	—	—	—
Phosphorus (mg/dL)	3.5-11.6	—	3.3-5.0	3.2-4.9	—	—	—	—
Potassium (mEq/L)	2.3-7.3	—	4.9-7.7	3.2-4.7	—	—	—	—
Protein, total (g/dL)	2.0-4.6	—	5.5-6.8	3.0-4.1	—	—	—	—
Albumin (g/dL)	0.1-2.3	—	—	—	—	—	—	—
Globulin (g/dL)	1.1-4.1	—	—	—	—	—	—	—

Continued

TABLE 3-7 Physiologic and Hematologic Values of Select Amphibians. (cont'd)

Measurement	African clawed frog (*Xenopus laevis*)	American bullfrog (*Rana catesbeiana*)	Australian common green tree frog (*Litoria caerulea*)	Australian white-lipped tree frog (*Litoria* infrafrenata)	Cuban tree frog (*Hyla septentrionalis*)	Leopard frog (*Rana pipiens*) ♂	Leopard frog (*Rana pipiens*) ♀	Tiger salamander (*Ambystoma tigrinum*)
Sodium (mEq/L)	111-134	—	107-114	104-108	—	—	—	—
Triglyceride (mg/dL)	57-555	—	—	—	—	—	—	—
Uric acid (mg/dL)	0.1-0.4	—	0.2-0.7	0.1-0.2	—	—	—	—

[a]Hematology is presently of limited diagnostic value because of the lack of normal data and the wide variation in hematologic and biochemical values according to sex, season, and state of hydration.
[b]For leukocyte totals and percentages for various species, refer to The Wildlife Leukocytes Web site at wildlifehematology.uga.edu.

TABLE 3-8 Blood Collection Sites in Amphibians[3,a]

Collection Site	Species Reported	Notes
Ventral abdominal vein	Anurans	Vessel present on midline along the ventral coelom, between sternum and pelvis; risk of hitting coelomic organs; visualization may be confirmed via transillumination of coelom in some species
Lingual plexus	Anurans	With mouth open, depress tongue to expose buccal surface of the oral cavity; lingual plexus can be visualized as superficial vessels; sedation may be needed in some species; safely used in frogs as small as 25 g, possible salivary contamination
Femoral vein	Anurans	Superficial vessel present along the medial aspect of the femur; runs parallel with femoral nerve; sedation may be needed
Heart	Multiple	Sedation recommended; aim needle at ventricle, allow heart to passively fill syringe to avoid collapsing ventricle; visualization may be assisted with ultrasound
Ventral tail vein	Urodelans	Similar to reptiles; caudal vein runs along the ventral caudal vertebrate and can be accessed via ventral or lateral approach; tail autotomy possible in some species
Facial vein/musculo-cutaneous vein[16]	Anurans (Ranidae)	Facial vein forms at the middle of the orbit and courses caudally to the angle of the jaw, turning into the musculo-cutaneous vein as it passes the caudal half of the tympanum; blood may be collected just rostral or just caudal to the tympanum; insert needle in rostrocaudal direction at 30° angle to the skin[16]

[a]Blood volume has been reported to vary by species or genus. In general, it is safe to collect 10% of the blood volume from healthy animals (approx. 1% of body weight). Clinical judgment should be used in collecting blood from sick or debilitated animals.

TABLE 3-9 Differential Diagnoses by Predominant Signs in Amphibians.[a]

Sign	Common Causes	Suggested Diagnostics[b]
Changes in skin color	Infectious agents: virus, bacteria, mycobacteria nodules, saprolegniasis, chromoblastomycosis, other mycoses, protozoa, myxosporeans, microsporidia, helminths (*Capillaroides xenopi*), leeches, fly larvae, other arthropods, fish lice, mollusks Noninfectious causes: toxicosis, hypothermia, hyperthermia, dehydration, desiccation, burn, frostbite, trauma, neoplasia, nutritional secondary hyperparathyroidism, xanthomatosis/hyperlipidosis, drug reaction	Biology review of species in question; husbandry review (diet, water quality tests, soil pH, temperature); skin scrapes (wet mount and stained); PCR tests for ranavirus and chytrid; skin and blood cultures; fecal parasite exams; plasma cholesterol and triglycerides; radiograph for skeletal density; plasma calcium and phosphorus; CBC and other plasma biochemistries
Changes in skin texture	Infectious agents: virus, bacteria, mycobacteria, mycoses, protozoa, myxosporeans, microsporidia, helminths, fly larvae, leeches, mites, ticks, fish lice, other arthropods, mollusks Noninfectious causes: toxicosis, hypothermia, hyperthermia, dehydration, desiccation, stress, trauma (especially rostral abrasion), neoplasia, normal (e.g., dorsal crests in European newts, egg brood patch of Surinam toad, nuptial pads in male anurans)	Biology review of species in question; husbandry review (diet, water quality tests, soil pH, temperature); skin scrapes (wet mount and stained); PCR tests for ranavirus and chytrid; skin and blood cultures; fecal parasite exams; CBC and plasma biochemistries

Continued

TABLE 3-9 Differential Diagnoses by Predominant Signs in Amphibians. (cont'd)

Sign	Common Causes	Suggested Diagnostics
Excess mucus production	Infectious agents: virus, bacteria, mycoses, protozoa, helminths, arthropods, mollusks Noninfectious causes: toxicosis (ammonia, nitrite, chlorine, chloramine, salt, nicotine), poor water quality (pH, hardness, supersaturation), stress (cagemate, escape behavior, inappropriate soil pH or composition), hyperthermia, trauma	Husbandry review (diet, water quality tests, soil pH, temperature); skin scrapes (wet mount and stained); PCR tests for ranavirus and chytrid; skin and blood culture; fecal parasite exams; CBC and plasma biochemistries
Fluctuant mass	Infectious agents: bacterial abscess, mycobacteria (rare), mycoses (rare), protozoal cyst, myxosporeans, helminths (e.g., immature trematodes and cestodes), subcutaneous leeches, fly larvae, mites, pentastomes Noninfectious causes: lymphatic blockage (e.g., gout), xanthomatosis, toxicosis, trauma, fluid overload, thermal injury, hypocalcemia, neoplasia, normal (e.g., active marsupium of *Gastrotheca* spp. females, water sacs of *Cycloderma rana*, distended lymphatic sacs of *Ceratophrys* spp.)	Biology review of species in question; husbandry review (diet, water quality tests, soil pH, temperature); aspirate (wet mount, stained, culture); fecal parasite exams; plasma uric acid, cholesterol, and triglycerides; radiograph for skeletal density; plasma calcium and phosphorus; skin and blood cultures; CBC and other plasma chemistries
Corneal opacity	Infectious agents: bacteria, mycoses, nematodes Noninfectious causes: scar, corneal lipidosis/xanthomatosis, trauma, chemical irritation, toxicosis, neoplasia	Husbandry review; slit lamp ophthalmic exam; culture and sensitivity; plasma cholesterol and triglycerides
Sudden death	Infectious agents: iridovirus, bacteria, chamydiosis, chytridiomycosis Noninfectious causes: toxicosis (ammonia, household pesticides, chlorine), electrocution, hypothermia, hyperthermia, trauma, gastric overload/impaction, stress, drowning, neoplasia	Biology review of species in question; husbandry review (diet, water quality tests, soil pH, temperature); PCR tests for ranavirus and chytrid; necropsy; physical exam of cagemates (include CBC, plasma biochemistries, blood culture, fecal parasite exams); consider euthanasia and necropsy of one or more cagemates
Weight loss	Infectious agents: bacteria, virus, chromomycosis, other mycoses, mycobacteria, coccidiosis, flagellate or ciliate overgrowth, helminths Noninfectious causes: heavy metal toxicosis (e.g., copper), chemical irritation (e.g., ammonia, chlorine, salt, pH), stress from inappropriate husbandry (e.g., environmental temperature too high, cagemate aggression), ocular disease with vision impairment, xanthomatosis	Biology review of species in question; husbandry review (diet, water quality tests, soil pH, temperature); skin scrapes (wet mount and stained); fecal parasite exams; PCR tests for ranavirus and chytrid; CBC; skin and blood cultures; plasma calcium, phosphorus, cholesterol, and triglycerides; radiograph for skeletal density; other plasma biochemistries

TABLE 3-9 Differential Diagnoses by Predominant Signs in Amphibians. (cont'd)

Sign	Common Causes	Suggested Diagnostics
Anorexia, inappetence	Infectious agents: iridovirus, Lucke's herpesvirus, other virus, bacteria, mycobacteria, chytridiomycosis, chromoblastomycosis, mucormycosis, protozoa, myxosporean, microsporidial, helminth, fly larvae, pentastomes, mites, ticks Noninfectious causes: inappropriate environment (e.g., substrate, temperature, illumination, photoperiod, humidity, lack of furnishings and hiding spots, inappropriate cagemates, too many cagemates or visible specimens in adjacent cages, activity in room), inappropriate feeding practices (e.g., wrong kind of food/prey, wrong size of food/prey, feeding at wrong times, too many prey items offered at one time), frequent handling or cage servicing, nutritional secondary hyperparathyroidism, hypocalcemia, toxicosis (e.g., copper, ammonia, chlorine), xanthomatosis, ocular disease with vision impairment, neoplasia, geriatric/senescence, normal (i.e., estivation or hibernation cues)	Biology review of species in question; husbandry review (diet, water quality tests, soil pH, temperature); skin scrapes (wet mount and stained); PCR tests for ranavirus and chytrid; skin and blood cultures; fecal parasite exams; plasma cholesterol and triglycerides; radiograph for skeletal density; plasma calcium and phosphorus; CBC and other plasma biochemistries
Bloating	Infectious agents: virus, bacteria, mycoses, mycobacteria, gastrointestinal nematodes Noninfectious causes: hypocalcemia (especially in hylid frogs), toxicosis, hypothermia, decomposition of ingesta (e.g., gastric overload, low or high temperatures), pneumocoelom (i.e., ruptured lung or trachea), gas supersaturation	Biology review of species in question; husbandry review (diet, water quality tests, soil pH, temperature); fecal parasite exams; PCR tests for ranavirus and chytrid; plasma calcium and phosphorus; radiograph; aspirate (wet mount, stained, culture); plasma biochemical analysis; ultrasonography; radiograph; skin and blood cultures; CBC
Hydrocoelom	Infectious agents: virus, bacteria, mycoses, mycobacteria, verminous granulomata, filarids, other helminths Noninfectious causes: toxicosis (e.g., heavy metal, chlorine, ammonia, insecticide, distilled or reverse osmosis water), hepatic failure, renal failure, hypocalcemia, xanthomatosis, gout, neoplasia (especially ovarian, hepatic, or renal), failure to oviposit, normal (e.g., ovulation)	Biology review of species in question; husbandry review (diet, water quality tests, soil pH, temperature); aspirate (wet mount, stained, culture); fecal parasite exams; PCR tests for ranavirus and chytrid; plasma biochemical analysis; ultrasonography; radiograph; skin and blood cultures; CBC
Cloacal prolapse	Infectious agents: helminths, protozoa, colitis/cloacitis (bacterial, fungal) Non-infectious causes: mechanical ileus, dehydration, gastric overload, intussusception, hypocalcemia, nutritional secondary hyperparathyroidism, constipation, physiologic behavior, iatrogenic (handling, sedation), straining with oviposition (females), neoplasia	Biology review of species in question; husbandry review (diet, water quality tests, soil pH, temperature); fecal parasite exams/ impression smear of prolapsed tissue; radiograph; ultrasonography; plasma biochemical analysis; CBC

Continued

TABLE 3-9 Differential Diagnoses by Predominant Signs in Amphibians. (cont'd)

Sign	Common Causes	Suggested Diagnostics
Lameness	Infectious agents: virus, bacteria, mycobacteria, mycoses, protozoa, myxosporeans, microsporidia, helminths, fly larvae, pentastomes, mites Noninfectious causes: nutritional secondary hyperparathyroidism, trauma, malnutrition (e.g., hypovitaminosis B), thiaminosis, hypervitaminosis D, gout, xanthomatosis/hyperlipidosis, toxicosis (especially insecticides), neoplasia, drug reaction	Husbandry review (diet, water quality tests, soil pH, temperature); radiograph; plasma calcium and phosphorus; plasma cholesterol and triglycerides; fecal parasite exams; CBC and other plasma chemistries
Spindly leg	Infectious agents: iridovirus, larval cestodes or trematodes, subcutaneous nematodes Noninfectious causes: nutritional secondary hyperparathyroidism, malnutrition (e.g., hypovitaminosis B, protein deficiency, iodine deficiency, trace mineral deficiency, diet of parents, outdated food or vitamin supplements), toxicosis (ammonia, chlorine, nitrites), water quality (pH, hardness, temperature), crowding, poor illumination, trauma, genetic, hybridization	Biology review of species in question; husbandry review (water quality tests, temperature); diet (inspect actual food items and supplements in original containers); PCR tests for ranavirus and chytrid; necropsy; physical exam of cagemates and parents; consider euthanasia and complete necropsy of one or more cagemates

[a]This is based on the previous author's (Dr. Kevin M. Wright) clinical impressions of the most common underlying etiologies for gross symptomology; a patient's differential list should be a comprehensive review of all potential etiologies regardless of likelihood. Edited by current authors.
[b]Suggested diagnostics are presented in prioritized order.

TABLE 3-10 Selected Disinfectants for Equipment and Cage Furniture.[40,a]

Batrachochytrium dendrobatidis

- Sodium hypochlorite (household bleach) 1% for 1 min contact time
- Ethanol 70% for 1 min exposure time
- Benzalkonium chloride 1 mg/mL for 1 min contact time
- Desiccation and exposure to 50-60°C (122-140°F) heat for 30 min
- Exposure to 1:1000 quaternary ammonium compound Quat-128 (Waxie Sanitary Supply, San Diego, CA; 800-995-4466; www.waxie.com) for 30 sec; this contains 6.8% didecyl dimethyl ammonium chloride (DDAC) as the active ingredient

Ranavirus

- Nolvasan (chlorhexidine) 0.75% for 1 min contact time
- Sodium hypochlorite (household bleach) 3% for 1 min contact time
- Virkon S 1.0% for 1 min contact time
- Desiccation and exposure to 60°C (140°F) heat for 15-30 min

[a]In order to increase efficacy of disinfectants, rinse all organic material and debris from the surface before applying disinfectants.

TABLE 3-11 Guidelines for Managing Pet Amphibians with Nematode Parasites.[59]

- Determine purpose of captive amphibian
  - Pet amphibians are often kept for different purposes than captive assurance colonies
  - Plan must be with owner's informed consent
- Assess current health and body condition score (BCS)
  - If unthrifty
    - Consider any nematode ova, larvae, or adults significant. Treat for nematodes appropriately in light of other clinical findings
  - If well-fleshed, score the fecal parasite exam
    - If diarrhea, blood, mucus, or visible nematodes are present at any stage of the fecal parasite examination, treat
    - If stool appears grossly normal
      - and there are $\leq$5 RBC/HPF or $<$1 WBC/HPF, parasites may not be significant
      - and there are $>$5-10 RBC/HPF or $>$1-5 WBC/HPF, parasites are likely significant, treatment may be indicated
      - or there are $>$5 strongyle larvae/HPF on direct or float, treat
  - Treatment of amphibians that are apparently healthy, eating well, and maintaining or gaining weight, should be done with caution despite the presence of a few nematode ova or larvae per high-power field on direct or flotation fecal parasite exams
  - If any amphibians in the collection appear unthrifty, there are mortalities with nematodes implicated, or there are otherwise unexplained mortalities, treat for nematodes
  - Monitor with regular direct fecal parasite exams to evaluate a shift in cytology and fluctuations in nematode ova and larvae; while there is often no correlation between reduction in nematode ova or larvae in feces and actual reduction in nematode numbers, improvements in BCS and weight often happen when the ova or larvae counts go down and the feces has $\leq$5 RBC/HPF and $<$1 WBC/HPF
  - Success is measured by an amphibian having a normal weight and BCS, producing normal-appearing feces, and exhibiting normal behaviors
  - With problematic pets, routine randomly collected feces should be assessed for parasites

TABLE 3-12 Amphibian Quarantine Protocols.[59]

Because of worldwide amphibian population declines and local extinctions, assurance colonies are being brought into captivity in hopes of preserving species for the future. The importance of these assurance colonies, and the possibility of future reintroduction efforts, makes proper quarantine and infectious disease testing paramount. In most cases, amphibians destined for use in reintroduction programs should remain in permanent quarantine to prevent introduction of novel pathogens. A 30-day quarantine is the minimum suggested time for quarantine of low risk amphibians, and moderate to high-risk animals should be quarantined for 60-90 days. Release from quarantine is predicated on interpretation of morbidity and mortality, appropriate testing to detect important diseases, and a healthy body condition score and normal physical examination prior to release. Any quarantine plan must have the owner's informed consent before implementation.

Husbandry

- Facilities and equipment
  - Ideally, each quarantine area is spatially separated from areas containing other animals. In addition, separate air-handling systems should exist for individual areas. Tools should be designated for use only in quarantine areas. Some facilities may employ shower-in/shower-out protocols, but at the very least boots, smocks/coveralls should be worn when servicing quarantine animal areas.
  - Enclosures

Continued

TABLE 3-12 Amphibian Quarantine Protocols. (cont'd)

- Enclosures should be escape-proof and made of non-abrasive, non-toxic material that is easy to clean and disinfect. Enclosures with spartan furnishings are easiest to monitor and maintain in quarantine situations; however, many animals will not thrive in such conditions. Critical husbandry requirements should always take precedence over other needs.
- Food
- Transmission of infectious disease through food animals is possible. In cases where this is of significant concern, it may be prudent to establish on-site breeding colonies of prey items and occasionally screen for various pathogens.

Quarantine Examination

- Physical examination can be facilitated via manual restraint, restraint in a clear container, or via anesthesia.
- To control the spread of *Batrachochytrium dendrobatidis*, new gloves should be changed after handling each patient. Nitrile gloves are preferred for their ability to kill zoospores on contact. Bare hands are preferred over rinsing the same pair of gloves between patients.[36]
- It is important to individually identify animals maintained in groups. Microchips and subcutaneous polymers can be placed; however, retention can be a problem. Charting of characteristic colors/patterns can be useful in some species (maintaining a database of digital photographs can be helpful in this regard). Toe-clipping has been used as a last resort, but is not recommended in zoological or private collections for humane and health reasons.
- Body weight and body condition scores should be assessed on arrival, periodically throughout quarantine, and immediately prior to release.

Diagnostic Testing

- Fecal flotation and direct examination
  - Quality samples can be obtained by placing the animal in a small container lined with damp, plain paper toweling overnight following a meal. It is best to assume wild amphibians are parasitized even though fecal exams can often be negative for the presence of parasites or ova.
  - Complete blood count and chemistry panel
  - A large percentage of amphibians are too small to safely take routine blood samples. Even larger specimens can present venipuncture challenges. See Table 3.8 for venipuncture sites in amphibians. Normal ranges for bloodwork parameters are not available for the vast majority of species which creates interpretive challenges.
  - Specific infectious disease testing
  - It is paramount that all amphibians entering quarantine (whether wild caught or captive bred) be screened for chytridiomycosis and ranavirus infection. Polymerase chain reaction tests are available for both pathogens, although chytrid fungus may also be detected via cytology of skin scrapings. Other specific pathogen testing will depend on individual circumstances.

Prophylactic Treatment

- Fluid therapy
  - Newly captured or shipped amphibians can be stressed and dehydrated. Amphibian Ringer's solution (see Table 3.6) can be used as a bath to help hydrate the animal and replenish solutes.
  - Deworming
  - It is impractical to impossible to completely clear most amphibians of parasites. Treatment is aimed at reducing overall parasite burden. Treatment should address the results of diagnostic testing, otherwise empirical therapy with broad-spectrum anthelminthics is recommended.
  - Treatment for chytridiomycosis
  - Animals brought into captivity from areas suffering local declines due to chytridiomycosis should always be prophylactically treated. In other situations, it is recommended to avoid treatment unless an infection is diagnosed to avoid development of resistance to available drugs.

TABLE 3-12 Amphibian Quarantine Protocols. (cont'd)

Maintenance and Hygiene

- A variety of disinfectants are available for use in amphibian applications. Care should be taken to choose a product that meets the disinfection needs but is not unsafe for the amphibians.
- Heat, desiccation, and ultraviolet light can be used, in some cases, to disinfect equipment and materials without the hazards associated with chemical use.
- Proper disposal of solid waste/water is paramount to avoid exposure of native amphibians in the area to novel pathogens. At the least, wastewater should only be discarded into a sanitary sewer and solid waste be deeply buried or transferred to a landfill. Best practices involve treating all waste that comes in contact with quarantined amphibians as potential biohazardous waste and disposing of accordingly. Wastewater and other materials should never be discarded into the environment in a manner where exposure to native amphibians is likely.

Infectious Disease Screening

There are a number of laboratories that will perform various tests to document exposure to or presence of chytrid fungus or ranavirus particles via PCR. It is up to the clinician to evaluate the tests run by the various laboratories and interpret the results accordingly. The following are contact information for laboratories that perform ranavirus and/or chytrid testing. This list is by no means conclusive, and contact information was verified as of September 16, 2016.

Amphibian Disease Laboratory
San Diego Zoo Institute for Conservation Research
15600 San Pasqual Valley Rd.
Escondido, CA 92027, USA
760-291-5472 or 760-291-5470
http://institute.sandiegozoo.org/resources/amphibian-disease-laboratory
Chytridiomycosis and ranavirus

Pisces Molecular
1600 Range St., Suite 201
Boulder, CO 80301, USA
303-546-9300
www.pisces-molecular.com
Chytridiomycosis

Research Associates Laboratory
14556 Midway Rd.
Dallas, TX 75244, USA
972-960-2221
www.vetdna.com
Chytridiomycosis

Zoologix
9811 Owensmouth Ave., Suite 4
Chatsworth, CA 91311, USA
818-717-8880
www.zoologix.com
Chytridiomycosis, ranavirus, and various *Mycobacterium* species

A more complete list of laboratories for *Batrachochytrium dendrobatidis* testing can be found at:
http://www.amphibianark.org/the-crisis/chytrid-fungus/
A more complete list of laboratories in various countries for ranavirus testing can be found through the Global Ranavirus Consortium at: http://www.ranavirus.org/resources/testing-labs/

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[a]Note: Pessier AP, Mendelson JR[40] remains an important and convenient source of information on amphibian medicine and is available free online.

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