Efficacy of ivermectin as an anthelmintic in leopard frogs

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Summary: Ivermectin administered cutaneously at dosages of 2 mg/kg of body weight eliminated nematode infections in leopard frogs. Three clinical trials were conducted. In the first trial, 5 groups of 11 frogs were given ivermectin IM at dosages of 0, 0.2, 0.4, 2, or 20 mg/kg. All frogs given ivermectin IM at dosages of 2.0 mg/kg or greater died. In trial 2, 44 frogs, allotted to 5 groups, were given ivermectin cutaneously at 0, 0.2, 0.4, 2, or 20 mg/kg. Cutaneously administered ivermectin was not toxic at dosages up to 20 mg/kg. In trial 3, nematode infections were eliminated in all 10 frogs treated cutaneously with ivermectin at 2.0 mg/kg.

Parasitism has been identified as an important cause of morbidity and mortality in captive herpetologic collections. The respiratory tract, intestinal tract, and dermis of amphibians are frequently infected with nematodes. Various anthelmintic regimens have been recommended for elimination of nematodes in amphibians: levamisole at 10 mg/kg of body weight, IM, fenbendazole at 10 mg/kg, PO, and ivermectin at 0.2 mg/kg, IM. Many species of frogs and toads are difficult to medicate adequately with oral or injectable preparations, because of their small size and excitable nature. The study reported here was instituted to assess the safety and efficacy of cutaneous administration of ivermectin in anurans. A clinical trial was performed to establish approximate toxic doses of ivermectin administered intramuscularly and cutaneously. Efficacy of ivermectin administered cutaneously to clear nematode infections was then assessed.

Materials and Methods

In the first trial, 55 wild Rana pipiens were randomly allotted into 5 experimental groups. Initial data included body weight and a body condition rating of 1, 2, or 3. Vigorous frogs with no visible lesions and minimal protuberances of pelvic bony structures were rated 1; alert frogs free of external lesions but with mild to moderate pelvic protrusion were rated 2; and frogs without external lesions but apparently slightly to moderately depressed or having moderate to severe pelvic protrusion or both, were rated 3. The 11 frogs in each experimental group were housed in 40 × 28 × 15-cm plastic sweater boxes, containing tap water to an approximate depth of 2 cm and plain white paper towel substrate. Frogs were maintained at approximately 24°C, and bedding was changed every 48 hours. Live crickets were offered to excess every 48 hours.

Group 1 was given 0.1 ml of propylene glycol injected intramuscularly in the caudal aspect of the thigh (semimembranosus and adductor magnus muscles). Groups 2, 3, 4, and 5 were given IM injections (into the caudal aspect of the right thigh) of ivermectin at 0.2 mg/kg, 0.4 mg/kg, 2 mg/kg, and 20 mg/kg, respectively. Injection volume was controlled to between 0.05 and 0.1 ml per injection by dilution of the ivermectin preparation with propylene glycol. Frogs were monitored daily for 14 days after injection.

In the second trial, 44 clinically normal Rana pipiens were randomly allotted into 5 groups. Frogs were group housed as in trial 1. The frogs in the first of the 5 groups were given 0.1 ml of propylene glycol administered cutaneously to the dorsal thoracic region. Group-2 frogs were given approximately 0.2 mg of ivermectin/kg applied to the dorsal thoracic region. Group-3 frogs were treated with approximately 2 mg of ivermectin/kg applied to the dorsal thoracic region. Group-4 frogs were given the same treatment as group-3 frogs except that the solution was administered on the right hind limb. Group-5 frogs were treated with 20 mg of ivermectin/kg cutaneously on the dorsal thoracic region.

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*IVomec injection for cattle, 1% sterile solution, Merck, Sharp & Dohme, Rahway, NJ.
previously described. The frogs were evaluated for 14 days.

In the third trial, fecal specimens were obtained from 35 individually housed, wild-caught, clinically normal *Rana pipiens*. Feces were stored in 10% formalin prior to coprologic analysis by ether extraction. Three of the frogs infected with nematodes were euthanatized and necropsied. The other 10 frogs with nematode eggs or larvae in their feces were treated with approximately 2 mg of ivermectin/kg applied cutaneously on the dorsal portion of the thorax. Frogs were housed individually and feces rechecked at 7, 14, 21, and 28 days.

**Results**

In the first trial, none of the group-1, -2, or -3 frogs died or became moribund during the 14-day study period; however, approximately one third to one half of the frogs from each group developed transient, mild to moderate hyperextension of the injected limb immediately after treatment. All frogs were clinically normal within 20 minutes. Two group-4 frogs developed flaccid paralysis between 10 minutes and 3 hours after treatment, and died within 24 hours. All group-5 frogs developed flaccid paralysis within 2 hours after treatment. Twenty-four hours after treatment, 9 group-5 frogs had died and 2 were unresponsive, with flaccid paralysis and profound bradycardia. These 2 frogs were maintained in approximately 0.5 cm of water with their heads propped up to keep their nares above water, and observed at least every 6 hours. Both animals remained comatose until death at approximately 4 and 5 days after treatment, respectively. In trial 2, none of the frogs died or developed signs of illness. In trial 3, 13 of the 28 fecal specimens contained parasite eggs or larvae 13 of which contained nematode eggs or larvae. Suitable fecal specimens could not be obtained from 7 frogs. Parasites from the 3 euthanatized frogs were tentatively identified as *Strongyloides* spp, *Oswaldecruzia* spp, and *Capillaria* spp. Results of fecal examinations from the 10 treated frogs were negative at weeks 1, 2, 3, and 4.

**Discussion**

Cutaneous application of ivermectin has the advantage of ensuring timely delivery of medication with minimal restraint and avoidance of injection trauma. Amphibians have an extremely thin epidermis, with capillaries penetrating close to the moist skin surface. Such physiologic adaptations to cutaneous respiration make amphibians more susceptible to absorption of environmental substances than other vertebrates.

Ivermectin is a macrocyclic lactone disaccharide, composed of a mixture of 22,23 dihydroavermectin B1A, and 22,23 dihydroavermectin B1. It functions by stimulating release of gamma-aminobutyric acid (GABA), a neurotransmitter, thereby interfering with nerve conduction. In vertebrates, GABA is a CNS neurotransmitter, and because ivermectin, at therapeutic doses, generally is incapable of penetrating an intact blood-brain barrier, there are no neurologic effects. Toxicity in vertebrates is believed to develop as a result of ivermectin penetration through the blood-brain barrier into the CNS, with subsequent increased GABA activity and neurologic malfunction. Cutaneous dosages of 20 mg/kg did not induce toxicosis. Manifestations of toxicosis became apparent when ivermectin was administered at the rate of ≥2 mg/kg IM. The paresis and paralysis observed with the 2 mg/kg and 20 mg/kg dosages were similar to ivermectin-induced toxicosis reported in tortoises, and suggests that toxicosis is mediated through inhibitory GABA neurons. The difference between the toxic doses when the drug is administered cutaneously vs IM is unexplained, but may be a result of incomplete cutaneous absorption. Our trials suggest that cutaneous administration of ivermectin at 2 mg/kg is a safe and effective treatment for nematode infections in *Rana pipiens*.

**References**


