Evaluation of the safety of ivermectin-praziquantel administered orally to pregnant mares

Patrick Mercier, DVM; Francisco Alves-Branco, DVM, MSc; Maria de Fatima Sapper, DVM, MSc; Christopher Roger White, DVM

Objective—To evaluate the safety of an orally administered ivermectin and praziquantel paste with regard to variables associated with clinical findings, parturition, lactation, maternal care, and neonate viability in pregnant mares.

Animals—40 pregnant mares.

Procedure—Mares were randomly allocated into treatment (n = 20) and control (20) groups and administered a placebo or 3 times the therapeutic dosage of ivermectin (0.6 mg/kg) and praziquantel (4.5 mg/kg) at 14-day intervals until parturition. Physical examinations were performed on mares and their foals after parturition (on postpartum days 30, 60, and 90) to identify any drug-related effects. As an aid in assessing general health, hematologic and serum biochemical analyses were performed monthly on the mares.

Results—In blood constituents, minor alterations that were not biologically important were observed. Reproductive performance was not affected by the unusual treatment duration or high dosage, although the drugs were administered during a crucial period of equine embryonic development (30 to 60 days). Neither adverse effects on mares nor abortions occurred. Follow-up evaluations of the foals for a 3-month period did not detect any abnormalities.

Conclusions and Clinical Relevance—Administration of the ivermectin-praziquantel paste appears to be safe in pregnant mares and their foals. (*Am J Vet Res* 2003;64:1221–1224)

Ivermectin, which is composed of at least 80% 22,23-dihydroavermectin B_{1a} and < 20% 22,23-dihydroavermectin B_{1b}, was the first avermectin to be commercialized and was approved for use in animals in 1981.[1,2] Its unprecedented potency and new mode of action quickly made ivermectin the treatment of choice for nematode and arthropod parasitism in cattle, sheep, goats, swine, and horses.[3]

Received February 28, 2003.
Accepted April 28, 2003.
From the Medical Department, Virbac (SA) Laboratories, 06511 Carros Cedex-France (Mercier); Consultorio Médico Veterinario, Bagé, Rio Grande do Sul, RS Brazil (Alves-Branco, Sapper); and Sao-Judas Tadeu, 575 Bagé, RS Brazil (White). Dr. Mercier's present address is Virbac S.A. 13ième Rue L.I.D., BP 27, 06511 Carros Cedex, France. Dr. Alves-Branco's present address is Consultorio Médico Veterinario, Rua José do Patrocinio, N°115 CEP: 96.415-500, Bagé-RS Brazil. Dr. Sapper's present address is Consultorio Médico Veterinario, Rua José do Patrocinio, N°115 CEP: 96.415-500, Bagé-RS Brazil. Dr. White's present address is Virbac do Brasil Laboratories, Av. Eng. Eusébio Stevaux, 1368 Jurubatuba, Sao-Paulo, SP Brazil.
Supported by Virbac (SA), France.
The authors thank M. V. Vasconcelos Camargos and P. S. Alves Branco Duarte for technical assistance.
Address correspondence to Dr. Mercier.

Praziquantel (2-cyclohexylcarbonyl [1,2,3,6,7, 11b] hexa-hydro-4*H*-pyrazin [2,1-a] isoquino-lin-4-one) is a potent anti-schistosomal compound and highly effective against trematodes and cestodes.[4] Praziquantel was introduced as a novel anthelmintic in 1975 and is presently the drug of choice for treatment of a wide range of veterinary and human nematode and cestode infections.[5]

The combination of ivermectin and praziquantel in a single product has given veterinarians and horsemen the opportunity to combat a wide range of equine parasites, including tapeworm infestations.

The purpose of the study reported here was to evaluate the clinical and reproductive performance effects (parturition, lactation, maternal care, and neonatal viability) of biweekly treatment with an orally administered anthelmintic paste at 3 times the recommended dosage through 1 gestation period in mares. Additionally, the study assessed the viability of neonates of the mares after the treatment period.

Materials and Methods

Horses and pregnancy assessment—Forty pregnant mares of pure Crioula breed were obtained from 1 stud yard. A rectal palpation was performed on each mare for confirmation of pregnancy, and periodic visual observations or rectal palpations were performed until parturition. Using a computer-generated randomization schedule, mares at different early stages of gestation were ranked by age and assigned to a placebo-treated control group (n = 20; mean ± SD age, 132.6 ± 51.4 months) or a treatment group (20; age, 127.2 ± 47.7 months). A numbered halter identified each mare. Depending on the date of prestudy parturition, the mares received vaccinations for equine herpesvirus, equine influenza virus, equine viral arteritis, tetanus, and equine rhinopneumonitis virus (killed virus) and were acclimated for approximately 14 days prior to treatment (mean, 196 days of pregnancy before parturition). The mares were housed together in fecal-contaminated pastures continuously during the study.

Treatment—Because this was a masked study, the products given to the horses were identified only by the letters A or B. At no time during the study period were investigators aware of the contents of product A (placebo paste) or B (ivermectin and praziquantel paste). On day 0, mares were weighed, and the first treatment was administered orally. Control horses received the placebo paste at a volume corresponding to the volume of ivermectin and praziquantel paste given at 3 times the normal dosage of each active ingredient (3X volume, 0.03 mL/kg). Treated horses received ivermectin and praziquantel paste at 3 times the recommended dosage (yielding ivermectin at 0.6 mg/kg and praziquantel at 4.5 mg/kg), and treatment was repeated at 14-day intervals for the entire duration of pregnancy until parturition.

A commercially available, nonmedicated basal diet of grain and roughage was provided for maintenance.

Clinical observations, physical examinations, and clinical pathology—Clinical observations were conducted on each mare once daily throughout the treatment period, including assessment of general appearance, behavior, attitude, and appetite. A complete physical examination was performed on each mare on day 90 of gestation, at foaling, and on days 30, 60, and 90 postpartum. As an aid in assessing general health before, during, and after the treatment period, blood samples were collected from a jugular vein in evacuated glass tubes on days 0 (before the first treatment), 28, 56, 84, 112, 140, 168, and 196. Blood samples were analyzed for hematologic parameters and serum biochemical values. Hematologic analyses were performed by use of an automated cell counter[a] via the impedance principle. Blood cell concentrations were confirmed by use of a laser counting technique, and serum biochemical values were measured by use of a semiautomatic method. Serum analyses included calcium, phosphorus, sodium, potassium, chloride, creatinine, glucose, total protein, total bilirubin, and urea nitrogen concentrations.

Clinical observations of each foal (at birth and days 30, 60, and 90 thereafter) included body weight at birth, gait, pupillary light reflex, cardiac auscultation, and an evaluation of conformation to include examinations of the nostrils, hard palate, spine, tail, anus, vulva, testicles, coat, limbs, and hooves.

Reproductive parameters—Gestation index (number of pregnant mares with live-born foals divided by number of pregnant mares), the number of foals born, and foal indices (number of live foals divided by number of mares that conceived) were measured on days 30, 60, and 90 after parturition.

Statistical analyses—The outcomes of the study (physical observations, blood parameters, pregnancy parameters, and adverse drug events) were examined separately. Treatment was the only explanatory factor that was considered. The time course of all hematologic and serum biochemical parameters was analyzed via ANOVA for repeated measures with group, time, and the interaction between time and group as fixed effects. If a significant ($P \leq 0.05$) interaction between group and time was detected, the nature of the interaction was studied. If the interaction was quantitative, the group effect in the model was interpreted; otherwise, within-time group effects were evaluated with a Student *t* test or Mann-Whitney *U* test. If there was a significant difference between groups on day 0, the parameter was transformed to

Table 1—Hematologic and serum biochemical values (mean ± SD [range]) in 20 control horses and 20 horses administered a combination of ivermectin (0.6 mg/kg) and praziquantel (4.5 mg/kg) at 14-day intervals during pregnancy

Parameter	Reference range	Before treatment		196 days after treatment	
		Control	Treated	Control	Treated
RBC ($\times 10^6$ cells/μL)	(6–12)	8.22* ± 0.61 (7.23–9.45)	8.64* ± 0.57 (7.81–10.4)	7.57 ± 0.77 (5.8–8.89)	8.36 ± 0.46 (7.56–9.64)
WBC ($\times 10^6$ cells/μL)	(6.000–12.000)	11.572 ± 2.062 (7.440– 16.60)	11.260 ± 1.695 [9.160–14.100]	12.663 ± 1.867 (9.860–16.000)	11.160 ± 1.626 [9.470–16.800]
Hemoglobin (g/dL)	(10–18)	12.88 ± 0.71 (11.6–14.4)	12.81 ± 2.35 (3.8–15.8)	12.20 ± 1.08 (9.22–13.9)	13.12 ± 0.73 (11.8–14.5)
Hct (%)	(32–48)	37.3 ± 1.94 (33.5–41.3)	38.33 ± 2.91 (31.7–45.4)	35.93 ± 3.29 (26.7–41)	38.69 ± 2.29 (34.9–42.4)
Neutrophils (%)	(30–75)	55.6 ± 10.82 (36.8–74.3)	56.02 ± 9.49 (35.8–80.2)	55.8 ± 13.62 (5.8–68)	56.21 ± 8.9 (38.9–70)
Eosinophils (%)	(1–10)	6.23 ± 2.34 (2.8–11)	6.61 ± 2.54 (3.8–12.9)	8 ± 2.59 (3.8–12.5)	6.14 ± 2.05 (2.8–10.3)
Basophils (%)	(0–3)	0.38 ± 0.44 (0.1–1.8)	0.19 ± 0.1 (0.1–0.4)	0.33 ± 0.26 (0.1–1.1)	0.28 ± 0.23 (0.1–1.1)
Monocytes (%)	(1–8)	4.1 ± 1.44 (1.6–6.9)	3.56 ± 1.1 (0.9–6.5)	4.81 ± 1.89 (1.5–9)	4.56 ± 1.96 (1.2–9.6)
Lymphocytes (%)	(25–60)	33.9 ± 11.3 (13.8–52.2)	33.6 ± 9.71 (10.9–53.9)	28.3 ± 5.43 (18.7–40.1)	32.3 ± 7.56 (12.6–43.1)
Platelets (cells/μL)	(100,000–600,000)	215.775 ± 84.806 (24.400–402.000)	241.800 ± 131.690 (136.000–755.000)	263.415* ± 67.236 (99.300–440.000)	222.945* ± 62.204 (79.900–324.000)
Glucose (mg/dL)	(62.2–114)	82.5 ± 6.29 (66–91)	82.0 ± 5.69 (71–93)	71.75 ± 6.75 (59–81)	71.80 ± 8.43 (52–88)
Creatine phosphokinase (U/L)	(34–165.6)	446.3 ± 118.37 (297–667)	481.4 ± 150.42 (270–885)	382.55 ± 134.39 (185–766)	402.4 ± 137.09 (194–780)
γ–Glutamyl transpeptidase (U/L)	(2.7–22.4)	17.55 ± 8.93 (10–490)	14.15 ± 7.42 (2–40)	12.50 ± 6.42 (6–33)	10.95 ± 4.31 (3–19)
Bilirubin (mg/dL)	(0.30–3)	0.59 ± 0.12 (0.37–0.85)	0.60 ± 0.16 (0.35–1.17)	0.79 ± 0.16 (0.56–1.1)	0.79 ± 0.12 (0.62–1.9)
Total protein (g/dL)	(5.7–7.9)	8.75 ± 0.68 (6.7–9.7)	8.48 ± 0.77 (7.2–10.1)	7.89 ± 0.91 (6.2–9.7)	7.36 ± 0.77 (5.8–9.2)
Urea (mg/dL)	(20–48)	49.15 ± 6.89 (37–64)	47.35 ± 6.49 (36–61)	50.14 ± 10.16 (32–77)	46.51 ± 7.04 (30–59)
Creatinine (mg/dL)	(0.9–2)	1.44 ± 0.14 (1.26–1.87)	1.44 ± 0.24 (0.63–1.89)	1.34 ± 0.30 (0.92–1.85)	1.24 ± 0.22 (0.89–1.94)
Na (mEq/L)	(133.3–147.3)	138.15 ± 2.23 (135–142)	138.45 ± 1.87 (136–142)	144.25 ± 2.61 (140–148)	144.70 ± 2.22 (140–149)
K (mEq/L)	(2.8–4.7)	3.93 ± 0.23 (3.5–4.3)	3.92 ± 0.18 (3.7–4.3)	4.60 ± 0.22 (4.2–4.9)	5.56 ± 2.64 (4–13.4)
Cl (mmol/L)	(97.2–110.1)	90.41 ± 19.04 (10.2–99)	94.75 ± 1.51 (91–98)	101.2 ± 2.70 (96–106)	100.95 ± 1.84 (98–104)
P (mg/dL)	(2.3–5.4)	3.86 ± 0.55 (2.8–4.7)	3.89 (± 0.61) [3.2–5.9]	5.48 ± 1.05 (3.4–8.4)	5.31 ± 0.73 (4.2–6.9)
Ca (mg/dL)	(10.4–13.4)	11.98 ± 1.34 (9.8–14.7)	11.99 ± 1.20 (9.2–13.7)	12.03 ± 0.89 (10–13.1)	12.22 ± 0.86 (10.4–13.6)

*Significant ($P < 0.05$) difference between groups.

compare the time course of the reduction percentage between day 0 and each time point. The percentage was calculated as follows: value at day_x – value at day_0/value at $day_0 \times 100$. The goal of the analyses was to identify a drug-related effect.

Results

Clinical observations—Administration of ivermectin and praziquantel paste to pregnant mares at 3 times the recommended dosage biweekly through 1 gestation period did not result in any adverse effects. The foals received a minimum of 4 physical examinations and did not have abnormal clinical signs or adverse events that appeared to be drug related. Mean ± SD foal body weights significantly ($P = 0.012$) differed between groups (foals of treated mares, 46.95 ± 5.4 kg; foals of control mares, 42.15 ± 6.05 kg).

Reproductive parameters—The gestation index in both groups was 1. The number of foals born and the foal indices on days 30, 60, and 90 were similar in the 2 groups.

Hematologic parameters and serum biochemical values—The RBC concentration in the control group on day 196 was significantly lower than in the treated group, whereas the WBC concentrations remained stable at approximately the same values throughout the study (**Table 1**). Although most hematologic parameters were within reference ranges for horses, the percentage of eosinophils was high on day 196 in the control group, compared with the treated group (8 and 6.14%, respectively). The creatine phosphokinase value recorded before and after treatment in both groups was approximately 3 times the baseline value.

Discussion

The active ingredients in the test product, ivermectin and praziquantel, have been widely used in humans. Adverse effects reported with ivermectin administration in humans are generally consistent with a mild reaction from its effect on microfilariae. These effects are generally transient and, if treatment is required, respond to analgesics and antihistamines.[6] Adverse effects (eg, abdominal discomfort and nausea) with praziquantel may be common but are usually mild and transient. Allergic-type reactions, such as fever, urticaria, and pruritic skin rashes, may also develop.[7] In our study, none of the pregnant mares had any clinical signs of adverse effects. Clinical signs of an acute toxicosis were first identified when horses were given ivermectin IM at 12 mg/kg (ie, 60 times the recommended dose).[8] Treated horses had signs of depression, ataxia, mydriasis, lower lip droop, and decreased respiratory rate. Egerton et al[9] reported transient impairment of vision, signs of depression, and ataxia in 5 horses and dehydration in 2 horses during the 5 days after oral administration of ivermectin paste at 10 times the recommended dose (2.0 mg/kg) for 2 consecutive days. At no time during our study were these types of adverse effects observed.

Blood variables, such as leucocyte concentrations, Hct, and protein concentrations measured by electrophoresis, may indicate parasitism, as reported by Ooms et al.[10] Velichkin[11] and Smith[12] reported that parasite-affected horses develop anemia with or without accompanying eosinophilia, lymphocytosis, or both. In our study, the minor alterations in blood constituents observed before and after treatment of the 40 pregnant mares appeared to be biologically unimportant and similar to those reported by Asquith and Kulwich.[13] On day 196, the high number of circulating eosinophils and anemia in control horses may have reflected a parasite infestation. It should be noted that the creatine phosphokinase values in our study were higher (before and after treatment) than the baseline values but were not attributable to pathologic conditions or muscle tissue damage. In our experience, these values may increase with muscular exertion alone but should return to reference range as the horse becomes more fit. Calcium, phosphorus, sodium, and glucose values within reference ranges have been reported in horses[14] given doses of ivermectin up to 12 mg/kg.

The major elimination pathway for ivermectin is fecal excretion.[15] Praziquantel is metabolized in the liver via cytochrome P_{450}.[16] The liver and kidneys of the horses reported here were not affected by drug administration under these special conditions and for this unusual period of time, as confirmed by bilirubin, γ–glutamyl transpeptidase, urea nitrogen, and creatinine values.

The effect of ivermectin on reproduction and breeding performance in mares and stallions has been investigated.[9] A group of mares was treated 6 or 7 times with ivermectin at 0.6 mg/kg (3 times the recommended dose) on about day 90 of pregnancy with no anatomic or functional defects in their progeny. The first 3 months of equine gestation are recognized as the most crucial to successful reproduction.[17] The equine embryonic period occurs in the first 30 to 60 days of gestation, during which the embryo develops the identifiable external features characteristic of the *Perissodactyla* taxonomic order.[18] The effect of ivermectin at 3 times the therapeutic dosage during equine fetal organogenesis was first investigated by McKissick et al[19] in 1987 by postnatal evaluation of ontogeny, and no teratogenic anatomic defects were discernible in the progeny of medicated mares. In our study, the tested product given orally at 3 times the therapeutic dosage to pregnant mares did not induce toxicosis in embryos or fetuses, and all foals born were structurally normal. In this safety study, 40 mares produced 40 healthy foals. The difference in foal body weights between groups at birth (foals of treated mares were 4.8 kg heavier) indicated that administration of the product to mares did not affect the growth of their foals and was not an abortifacient.

[a]Model Cell-Dyn CD 3500 CS, Abbott Medical Instruments, Chicago, Ill.

References

1. Chabala JC, Mrozik H, Tolman RL, et al. Ivermectin, a new broad-spectrum antiparasitic agent. *J Med Chem* 1980;23:1134–1136.
2. Egerton JR, Birnbaum J, Blair LS, et al. 22-23 Dihydroavermectin B1, a new broad-spectrum antiparasitic agent. *Br Vet J* 1980;136:88–97.
3. Campbell WC, Fisher MH, Stapley EO, et al. Ivermectin: a potent new antiparasitic agent. *Science* 1983;221:823–828.

4. Andrews P, Thomas H, Pohlke R, et al. Praziquantel. *Med Res Rev* 1983;3:147–200.

5. Gönnert R, Andrews P. Praziquantel, a new broad-spectrum anti-schistosomal agent. *Z Parasitenkd* 1977;52:129–150.

6. Martindale W. Ivermectin. In: Reynolds JEF, ed. *The extra pharmacopoeia.* 30th ed. London: The Pharmaceutical Press, 1993;43–44.

7. Martindale W. Praziquantel. In: Reynolds JEF, ed. *The extra pharmacopoeia.* 30th ed. London: The Pharmaceutical Press, 1993;52–53.

8. Campbell WC, Benz GW. Ivermectin: a review of efficacy and safety. *J Vet Pharmacol Ther* 1984;7:1–16.

9. Egerton JR, Seward RL, Robin B. Ivermectin as an antiparasitic agent for horses, in *Proceedings.* 22nd World Vet Cong, MSD AGVET Symp Recent Dev Control Anim Parasites 1983;49–55.

10. Ooms L, Oyaert W, Muylle E, et al. Electrophoresis as an aid to the diagnosis of helminthiasis and especially of verminous aneurysms in horses. *Vlaams Diergeneeskundig Tijdschrift* 1976;45:290–299.

11. Velichkin PA. Nodular trichonematosis of the large intestine in stud horses. *Izv Moskov Zootekhnich Instit Konevod* 1952;7:18–25.

12. Smith HJ. Strongyle infections in ponies II. Re-infection of treated animals. *Can J Comp Med* 1976;40:335–340.

13. Asquith RL, Kulwich R. Safety and therapeutic activity of ivermectin as an equine anthelmintic. *Equine Vet Sci* 1981;1:18–20.

14. Leaning WHD. The efficacy and safety evaluation of ivermectin as a parenteral and oral antiparasitic agent in horses, in *Proceedings.* Am Assoc Equine Pract Annu Meet 1983;29:319–328.

15. Lee Chin SH, Lu AH. Metabolism and tissue residues. In: Campbell WC, ed. *Ivermectin and abamectin*. New York: Springer-Verlag Inc, 1989;131–143.

16. Masimirembwa CM, Hasler JA. Characterisation of praziquantel metabolism by rat liver microsomes using cytochrome P450 inhibitors. *Biochem Pharmacol* 1994;48:1779–1783.

17. Jones WE. *Genetics and horse breeding.* Philadelphia: Lea & Febiger, 1982;460–490.

18. Bergin WC, Gier HT, Frey RA, et al. Developmental horizons and measurements useful for age determination of equine embryos and fetuses, in *Proceedings.* 13th Annu Conv Am Assoc Equine Pract 1967;179–196.

19. McKissick GE, Sutherland IH, Foix J, et al. The safety of ivermectin administered orally to pregnant mares. *Equine Vet Sci* 1987;7:357–367.