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Evaluation of clinical safety and anthelmintic efficacy of aurixazole administed orally at 24 mg/kg in cattle

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ABSTRACT

The current study evaluated, *in vivo,* the clinical safety and the anthelmintic efficacy of 24% aurixazole (24 mg/kg), administered orally, in bovines. Two experiments were conducted: the first one evaluating the clinical safety of 24% aurixazole (24 mg/kg) in cattle, and a second one evaluating the anthelmintic efficacy of aurixazole (24 mg/kg) against gastrointestinal nematodes on naturally infected cattle. Based on the results of clinical safety, no alterations on clinical and haematological signs and on the biochemical values obtained in animals treated orally with aurixazole 24 mg/kg were observed. Regarding the results of reduction or efficacy, obtained by eggs per gram of faeces (EPG) counts, the formulation of aurixazole reached values superior to 99% (arithmetic means) in all post-treatment dates. In two occasions, this formulation reached maximum efficacy (100%). Comparing these results with the reduction percentages obtained by EPG counts, it is possible to verify that the values obtained by all three formulations were compatible with the efficacy results. Aurixazole reached maximum efficacy (100%) against *Haemonchus placei*, *Cooperia spatulata* and *Oesophagostomum radiatum*. Against *Cooperia punctata*, this formulation reached an efficacy index of 99.99%. Regarding aurixazole, no specific trials were conducted on the field in order to evaluate the behaviour of this molecule against helminths that are resistant to other molecules, specially isolated levamisole and disophenolat. Due to this fact, future studies will be necessary to assess the effectiveness of aurixazole against strains of nematodes that are resistant to levamisole and disophenolat, but the results of clinical safety and efficacy described in this study allow us to conclude that the aurixazole molecule, concomitantly with other measures and orally administered formulations, can be another important tool in the control of nematodes parasitizing bovines.

1. Introduction

The constant population growth has stimulated beef production in order to meet the demand of protein consumption. Consequently, it is possible to observe a more intensive exploration, increasing the number of animals per acre. This leads to greater sanitary problems, including the gastrointestinal nematodes parasitizing bovines (Borges et al., 2008).

Nematode-infected cattle presented slow growth, decay in beef and milk production, and a low reproductive performance, leading to an increase in the losses observed by producers. Even though bovines can present helminth infections in all ages, young animals are more susceptible to these parasites (Stromberg et al., 2012). The control of such parasitic diseases in cattle still depends, almost exclusively, on the use of anthelmintics. According to Charles and Furlong (1996), among the recently available chemical groups, macrocyclic lactones are the ones being most used in cattle helminth control in Brazil, followed by imidazoles and benzimidazoles. However, its indiscriminate and incorrect use leads to the selection of resistant nematode strains (Soutello et al., 2007).

In an attempt to find formulations that are active against bovine nematodes, a new molecule named aurixazole (chemically called disophenolate of levamisole = 2,6-diiodo-4-nitrophenolate of levamisole), was originally developed by the 'Ouro Fino Agronegócio' company, the producer of this compound that is not yet available in a commercial formulation. This company is located in Ribeirão Preto city, São Paulo State, Brazil. The disophenol, which chemical structure is denominated 2,6-Diiodo-4-nitrophenol, belongs to the group of phenolic substitutes, and it was introduced in the market as a parenteral anthelmintic, admin-

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istered subcutaneously in dogs for the treatment against *Ancylostoma* spp. (Wood et al., 1961). These compounds, when administered in cattle by subcutaneous route at the dose of 10 mg/kg, interfere in the respiratory metabolism of helminths, blocking the production of energy by inhibiting mitochondrial oxidative phosphorylation, preventing the use of oxireduction reactions for the production of adenosine triphosphate (ATP). This product has reduced safety index and it should be applied on the due day and time under milder temperatures, in rested animals. Levamisole, which chemical structure is (S)-6-Phenyl-2,3,5,6-tetrahydroimidazole[2,1-*b*][1,3]thiazole, is an anti-nematode chemical belonging to imidazothiazole derivative groups (Spinosa et al., 2006). This group is used in cattle by subcutaneous rout, at the dose of 9.4 mg/kg and it acts mainly in the neuromuscular coordination of parasites, as a nicotinic cholinergic agonist. They penetrate the parasite through the cuticle and bind to acetylcholinergic neurotransmitters, causing excessive hyperpolarization of the post-synaptic membrane and spastic paralysis of the parasites (Martin, 1993). As to disophenol, the levamisole is a drug with reduced safety index, and even at therapeutic doses, the animals may exhibit excitement and drooling.

As a result of the great need to develop new active principles for the control of nematodes in cattle, this study evaluated the clinical safey at therapeutic dosage and the anthelmintic efficacy of aurixazole administed orally at a dosage of 24 mg/kg in cattle. The choice of the 24 mg/kg dosage was made because this value was considered effective based on the previous results obtained by the company regarding this molecule, and this dosage showed a positive correlation between the cost and benefit for the costumer.

2. Materials and methods

2.1. Experiment 1 – evaluation of clinical safety at therapeutic dose

2.1.1. Trial location and selected animals

The study was only conducted at therapeutic doses. This experiment was conducted in IPESA – Institute of Researches in Animal Health (Bananal Farm), located in the city of Formiga, Minas Gerais state, Brazil. Twenty adult, male, crossbreed (*Bos taurus* × *Bos indicus*) bovines, with ages ranging from 22 to 36 months, were used. All animals were kept in grazing paddocks (*Brachiaria decumbens*), receiving mineral supplementation and water *ad libitum*. The animals that took part in this study had no physical contact with other bovines.

All of the selected animals were clinically healthy, and they were evaluated by means of physical parameters on the pre-selection period (45 days before treatment) and 7 days before treatment (D–7). These animals went through a period of 90 days without the administration of any other drugs, especially anti-parasitic compounds.

The 20 adult bovines were divided into two groups with 10 animals each. Ten animals were kept as controls (saline solution, administered orally), and another 10 were treated once orally with 24 mg/kg aurixazole.

2.1.2. Clinical evaluation

For each animal, an identification record was created, containing the bovine's number, age and body weight. Clinical observations were conducted on day 7 before treatment, on day zero (before treatment), and on the 1st, 2nd, 3rd, 7th and 14th post-treatment days (PTD). At these dates, clinical parameters were verified (cardiac and respiratory frequencies and rectal temperature), as well as eye abnormalities and the cardiovascular, respiratory, muscular-skeletal, integumentary (skin, hair and hooves), gastrointestinal, nervous, lymphatic and urinary systems. During the experiment, all animals were also evaluated regarding the presence of clinical signs of systemic poisoning, such as ataxia, siallorhea, prostration, excitement, convulsions, dysphonia, diarrhoea, dyspnoea, jaundice, cough, head tremors, skin tremors, hyperaesthesia, among others.

2.1.3. Haematological and biochemical exams

Complementary laboratory exams were performed in all animals on days –7, zero (before treatment), and on days 3, 7 and 14 post-treatment. For haematological exams, 10 ml of blood was collected in vials containing EDTA as anticoagulant. The samples were stored and transported in ice (from 0 to 4 ° C) to the Endomed Clinical Pathology Lab located in Jabotical city, where the following exams were conducted: erythrocyte count, globular volume, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), counts of platelets and white blood cells, including differential. Analyses were performed using ABX vet® equipment.

For the biochemical exams, 10 ml of blood was collected in vials without additives, in order to separate the serum. Samples were analysed regarding urea (enzymatic method using the Katal Biotecnológica Indústria e Comércio Ltda. – Belo Horizonte, MG), creatinine (modified Jaffé method using the Katal Biotecnológica Indústria e Comércio Ltda. – Belo Horizonte, MG), alkaline phosphatase (AP, Bowers & McComb modified method using the Labtest Diagnóstica S.A. – Lagoa Santa, MG), aspartate amino transferase (AST, optimized UV method – IFCC using the Wiener Lab. – Rosario, Argentina), gamma glutamyl transferase (GGT kinetic method using the Katal Biotecnológica Indústria e Comércio Ltda. – Belo Horizonte, MG using using the Katal Biotecnológica Indústria e Comércio Ltda. – Belo Horizonte, MG) and creatine kinase (CK, kinetic UV method – IFCC usig the Labtest Diagnóstica S.A. – Lagoa Santa, MG). Readings were performed in an automatic spectrophotometer using the automatic biochemical analyser, COBAS MIRA S, Roche do Brasil – São Paulo, SP.

2.2. Experiment 2 – anthelmintic efficacy in naturally infected animals

2.2.1. Study animals

Experiment II was performed in the same farm, located in the centre-west region of Minas Gerais, Brazil, in which populations of *Haemonchus placei*, *Cooperia punctata*, *Cooperia spatulata, Trichuris discolor* and *Oesophagostomum radiatum* had already been described as resistant to macrocyclic lactones (Borges et al., 2008; Lopes et al., 2009, 2013). Thirty-two naturally infected calves aged 6 to 12 months were selected through individual counts of nematode eggs per gram of faeces (EPG ≥ 500) using the method described by Gordon and Whitlock (1939). The animals were randomly sorted into four groups of eight bovines each, by means of three consecutive EPG counts (days –3, –2 and –1). Blocking was based on the mean counts of eggs per gram (EPG) of Strongilidea in three consecutive days (–3, –2, –1). There were 8 blocks consisting of 4 animals each. Within each block, animals were randomly allocated to treatment. After the random draw, eight animals were kept as control (saline solution, administered orally), another eight were treated orally with 24 mg/kg of aurixazole (*Disophenolate* of levamisole = 2,6-diiodo-4-nitrophenolate of levamisole, produced by Ouro Fino Agronegócio) in a volume of 1 ml/10 kg of body weight, another eight were treated orally with 7.5 mg/kg of fenbendazole (4%; Panacur® Suspension; MSD Animal Health; Brazil) and another eight animals were treated orally with 5 mg/kg of albendazole (10%; Valbazen® 10%; Cobalt; Zoetis; Brazil).

All thirty-two animals of this second study were transferred and held in suspended freestalls at Animal Health Research Center