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Evaluation of melarsamine hydrochloride (Cymelarsan®) efficacy for the treatment of dourine nervous form on experimentally infected ponies

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The causative agent of dourine, *Trypanosoma equiperdum*, may cross the brain-blood-barrier leading to the apparition of nervous signs in infected horses. This location participates to the protection of the parasite from most (if not all) existing chemotherapies. In this context, the OIE terrestrial code considers dourine as a non-treatable disease and imposes to practice a stamping-out policy for affected animals to recover a country free status. A recent study suggests that melarsamine hydrochloride has the capacity to cure infected horses (*Hagos et al., 2010*). Still, the perspective to authorize Cymelarsan® for dourine treatment remains under debate since its capacity to eliminate the parasite from nervous system central is not proven. The goal of this study is to evaluate the capacity of Cymelarsan® to eliminate *T. equiperdum* from the overall organism of infected ponies including cerebrospinal fluid. For this purpose, four female Welsh ponies were infected with the *T. equiperdum* OVI reference strain. Parasites were observed in the cerebrospinal fluid of the four ponies between 5 to 19 days after detection in the blood, thus validating our dourine nervous stage model. Two ponies were treated one day after observation of the parasites in the cerebrospinal fluid (early treatment) and two were treated after apparition of nervous clinical signs (late treatment). Following one administration of Cymelarsan® (0.5 mg/kg), *T. equiperdum* was cleared from the blood of the two lately treated ponies but a massive infection was observed in cerebrospinal fluid. Thereafter, a daily repeated Cymelarsan® (0.5 mg/kg) treatment was administrated to one of the lately treated ponies (n=5 injections) and to the two early treated ponies (n=6 injections). Following this treatment, parasites were cleared from the blood circulation of all the ponies but a massive *T. equiperdum* infection was observed in the cerebrospinal fluid one of the lately treated pony and not of the two early treated animals. As a conclusion, the Cymelarsan® treatment failed to cure the two ponies at a single dosage but efficacy of a repeated treatment can be supposed depending on the stage of the disease. Further experimentations are ongoing in order to confirm these results.

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Evaluation of two methods for the diagnosis of equine gastric habronemosis caused by *Habronema muscae*

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Equine habronematidosis is caused by nematodes of the family Habronematidae and occurs clinically as gastric, cutaneous and pulmonal forms. Causative agents are Habronema muscae, H. microstoma and Draschia megastoma. Despite their world-wide distribution of these nematodes habronematidosis has drawn only little attention to veterinarians. One of the reasons is the difficulty to detect exogenic stages with routine diagnostic methods. The aim of our study was to compare the efficacy of a modified Mertiolate-Formaldehyd-Concentration (MFC) method for direct detection of Habronema eggs with a xenodiagnostic method where the parasite undergoes a partial development in housefly larval stages grown on horse faeces. In a first step, out of 6 visited places a suitable horse farm was selected to carry out our examinations. For this, muscid flies were caught and examined for the presence of Habronema larvae. Infected flies were found in 5 of the 6 farms in prevalences between 8 and 25%. The main trial was carried out with faeces of 33 horses of a riding school in which the prevalence of Habronema larvae in houseflies was 12%. Horse faecal samples were taken twice with an interval of 3 weeks. Three grams of faeces were used for the MIFC method that includes mixing the sample with 10 ml merthiolate-formaldehyd-solution and pouring the solution though a sieve (100 µm mesh) into a falcon tube. After adding of 2 ml of ethyl-ether and mixing the sample was centrifuged for 1 min at 241 g. Contrary to the classical MIFC method that uses iodine we used methylen blue as dye. Three drops of the stained sediment were transferred on a slide and examined at a magnification of 200 times. For the xenodiagnostic method, 50 g of the faeces were put for 2 - 4 h in a cage containing 500 adult house flies to allow the insects to deposit their eggs. The faecal sample was then stored at 26oC in a plastic container closed with a soft facial tissue. Flies that hatched 2-3 weeks later were immobilized by cold and were examined under a stereoscopic microscope for the presence of Habronema larvae. The first feacal check with the MFC method gave 5 positive results while the xenodiagnostic method with the subsequent samples diagnosed 17 positive horses. In 3 samples flies did not develop. A repeated examination 3 weeks later showed Habronema eggs in 17 samples. The xenodiagnostic method confirmed the 17 positive horses of the first examination and found 3 additional samples. As a result of our trial we can conclude that the MFC method can be used to confirm suspected cases of gastric H. muscae infection. However, not all horses excrete sufficient egg numbers all the time. This makes it necessary to examine a further sample. The more time-consuming xenodiagnostic method is more precise and can be used as gold standard.

Posters

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Housefly larvae of all stages can become infected with *Habronema muscae*

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The larval stage of equine stomach worm, Habronema muscae, had been known 50 years prior to the description of the adult nematode. As a result of early life cycle studies carried out with house fly (Musca domestica) larvae grown on horse faeces in the first half of the last century it was found that the helminth larvae invade cells of the adipose tissues of the maggot and it was concluded that the development of parasite and fly is synchronized in a way that the infective nematode larva is fully developed when the adult fly emerges. The objective of this research was to find out which developmental stage of house fly larva is susceptible to H. muscae infection. For this, M. domestica eggs were collected from a moist artificial breeding substrate (wheat bran, alfalfa flour and yeast) and transferred onto faecal samples of a horse with gastric habronemosis. Between day 2 (group D 2) and day 11 fly (group D 11) larvae were reisolated and placed in containers filled with artificial breeding substrate. The experiment was conducted in the laboratory at 24-26 °C. Under these conditions 1st and 2nd stage

maggots occurred 24 and 72 hours after oviposition, respectively. First 3rd stage maggots appeared one day later. Pupation started on day 8 after oviposition and most of the adult flies emerged between day 13 - 16. Altogether 386 freshly hatched flies were dissected and examined for nematode larvae. The average number (abundance) of Habronema larvae per fly rose steadily from 1.75 in group D 2 to more than 25 in groups D 10 and D 11. In the positive control group where fly larvae underwent their whole development on horse faeces parasite abundance equaled to 34.7, with a maximum burden of 60. Contrary to this, the development rate of flies from egg to imago declined from 74% in the group D 2 to 25% in group D 11. Flies that emerged from samples of groups D 9 - D11 and of the positive control were 30% shorter compared to those of the uninfected control group. There was no difference in parasite abundance between male and female flies. First stage Habronema larvae were seen in the intestine of fly maggots at day 2. Second stage nematode larvae of sausage shape remained in the adipose tissues of fly larvae with little changes in growth. The moult into the 3rd infective larval stage of the nematode only took place in the fly pupa. Out of 325 infected flies, nematode larvae were seen in caput, thorax and abdomen of 279, 243 and 284 individuals, respectively. The highest density of Habronema larvae in freshly hatched flies was found in the abdomen. As a result of our observation we can conclude that all larval M. domestica stages (1st, 2nd and 3rd instars) can become infected with H. muscae larvae and that the exposure to nematode eggs has a negative influence on survival and development of M. domestica.

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Anthelmintic resistance in equine nematodes in Argentina

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In Argentina, as many countries of the world, the most prevalent intestinal horse nematodes are small strongyle species or cyathostome group and presently there are practically just two clases of anthelmintics to control these parasites, benzimidazoles and macrociclic lactones. Pyrantel, a comom drug to control horse parasites in other parts of the world has practically not been used in Argentina. The gold standard for evaluation of the anthelmintic susceptibility of strongyle infection is the Feacl Egg Count Reduction Test (FECRT) which determine the average reduction in nematode egg count following treatment of a group of horses by obtaining quantitative egg counts on the day of treatmente and aproximately two weeks later. According to the FECRT populations of cyathostomins resistant to benzimidazoles are widespread in the country [1],[2] with more than 80 % of the farms studied showing anthelmintic resistance to these drugs . Despite these reports, dissemination of this information among equine veterinarians and owners has been poor and the use of benzimidazoles to control these parasites is still common. There is not report of cyathostome populations resistant to macrocyclic lactones despite over 30 years of use of these drugs in the country. However recently there are some field evidences that a shortened egg reaparence period appears to be emerging after ivermectin treatments. With respect to *Parascaris equorum*, wich is considered the most important parasitic pathogens of foals, during 2006 there have been two reports of suspected ivermectin resistance based on FECRT but they have not yet been confirmed with controlled efficacy studies. The clinical sigificance of anthelmintic resistance in cyathostomes or *Parascaris equorum* is currently unclear. Due to the relatively low pathogenicity of most cyathostomin infections, the anthelmintic resitance impact should be considered when this issue is discussed but the use of benzimidazoles should be avoided unless controls are carried out after treatment to determine its effectiveness. In contrast to the cyathostome, failure to control *P. equorum* with macrocyclic lactones, would lead to severe clinical disease with diarrhoea, gastrointestinal impaction and ocasionally perforation or ruptura of small intestine. Equine veterinarians should assess the presence of anthelmintic resistance in the farms before establishing a parasite control program. The FECRT, despite its limitations, should be used at least annually to monitor any program they institute on a farm for rational and sustainable use of anthelmintics.

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Small strongyles (cyathostomes) and benzimidazoles. Persistance of status of resistance after nine years without the use of these drugs and efficacy of ivermectin about this parasite population

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Background: in June 2005 was documented for the first time in Argentina (Santa Fe province) the presence in a horse farm of cyathostome populations resistant to benzimidazole [1] .From then on, the use of this chemical group was supplanted by ivermectin at this facility. The horse herd was relatively stable and closed and a new study was conducted in June 2014 to determine whether the absence of use of benzimidazoles may have affected the susceptibility of nematodes to this drug and compare the efficacy of ivermectin. Materials and Methods: twelve horses of 4-9 years of age were treated with mebendazole (6.25 mg / kg oral) and then re-treated twelve days later with ivermectin (0.2 mg / kg oral). The number of eggs/g feces eliminated (epg) was determined before and after treatment to perform a Fecal Eggs Count Reduction Test (FECRT) to evaluate clinical efficacy of the anthelmintics, using the following formula : % efficacy = (1-T2 / T1) x 100 , where T1 and T2 represent the average epg post and pretreatment respectively [2]. Results and discussion: after treatment with mebendazole and ivermectin, the FECRT was 40.53% and 100% respectively. The cyathostome population studied remained resistant to benzimidazoles after nine years without the use of benzimidazoles. These observations confirm that once resistance develops in a population of nematodes, the chances of