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

J. Cauchard1, D. Carnicer1, A. Madeline1, E. Guitton2, A. Giraudet1, P. Büscher3, L. Hébert1, C. Lauzier1

1 ANSES, Dozulé Laboratory for Equine Diseases, Bacteriology and Parasitology Unit, 14430 Goustranville, France; 2 Institut National de Recherche Agronomique, Tours, France; 3 Ecole Nationale Vétérinaire d’Alfort, Maisons Alfort, France; 4 Institute of Tropical Medicine, Department of Biomedical Sciences, Nationalstraat 155, B-2000 Antwerp, Belgium

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 of the parasite from most (if not all) existing chemotherapies. A recent study suggests that melarsamine hydrochloride has the capacity to cure infected horses (Hagas 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 experiments are ongoing in order to confirm these results.

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

S.M. Toenges1,2, R.K. Schuster3, S. Sivakumar1

1 Central Veterinary Research Laboratory, Dubai, UAE; 2 Beuth Hochschule für Technik, Berlin, Germany

Equine habronematisosis 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 habronematisosis 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 xenodiagnostics 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 MFC method that includes mixing the sample with 10 ml mertiolate-formaldehydsolution 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 MFC 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 xenodiagnostics 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 26°C 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 faecal check with the MFC method gave 5 positive results while the xenodiagnostics 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 xenodiagnostics 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 xenodiagnostics method is more precise and can be used as gold standard.

Posters

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

R.K. Schuster1, S. Sivakumar1

1 Central Veterinary Research Laboratory, Dubai, UAE

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 helmith 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 1th and 2nd stage