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Short communication

Melarsomine hydrochloride (Cymelarsan®) fails to cure horses with *Trypanosoma equiperdum* OVI parasites in their cerebrospinal fluid

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ABSTRACT

The aim of this study was to evaluate the ability of melarsomine hydrochloride (Cymelarsan®) to cure horses suffering from a nervous form of dourine, a sexually-transmitted disease caused by *Trypanosoma equiperdum*. The recently described experimental model for assessing drug efficacy against horse trypanosomosis allowed us to obtain eight horses (Welsh pony mares) infected by *T. equiperdum* with parasites in their cerebrospinal fluid. The Cymelarsan® treatment evaluated consisted of the daily administration of 0.5 mg/kg of Cymelarsan® over 7 days. Two control horses remained untreated, three horses received the treatment 36 days p.i. and three horses received the treatment 16 days p.i. Following treatment, we observed parasite clearance in blood, stabilization of rectal temperature and a relative improvement in the mean packed cell volume levels for all treated horses. However, live parasites were later observed again in the CSF of all treated horses. Our results indicate the inability of Cymelarsan® to reach *Trypanozoon* located in the central nervous system of infected horses and thus discourage the use of Cymelarsan® to treat animals suffering from a nervous form of dourine.

1. Introduction

Dourine is a sexually-transmitted parasitic disease caused by *Trypanosoma equiperdum* that affects horses and other members of the Equidae family (Zablotskij et al., 2003). It is generally considered that *T. equiperdum,* transferred during coitus, first penetrates mucous membranes, and then invades tissues, blood, and lymph, leading to clinical signs such as anemia, fever, and weight loss. *T. equiperdum* parasites can subsequently invade the cerebrospinal fluid (CSF) and the central nervous system (CNS), leading to nervous clinical signs such as incoordination and hind limb paralysis, and eventually cause the death of the infected animals (Brun et al., 1998; Scacchia et al., 2011).

Trypanosoma equiperdum belongs to the subgenus *Trypanozoon* that also includes the agents of nagana (*Trypanosoma brucei*) and surra (*Trypanosoma evansi*) (Carnes et al., 2015). Different drugs have been reported to be effective against *Trypanozoon* parasites (Brun et al., 1998), but it is generally considered that the capacity of the parasites to reach the CNS of its hosts protects them against the trypanocidal activity of most (if not all) existing drugs and participates in relapse following treatment (Jennings and Gray, 1983). These considerations led the World Organisation for Animal Health (OIE) to recommend, in its Terrestrial Animal Health Code, the slaughtering of dourine-infected animals due to the frequent relapses observed after treatment (OIE, 2013). To date, the adoption by the authorities of a treatment strategy for dourine-infected horses remains impaired by the lack of information on the capacity of trypanocides that are recognized as effective *in vitro* to actually cure horses suffering from a nervous form of the disease. This is especially true for melarsomine (Cymelarsan®), which has been shown to be effective against *T. equiperdum in vitro* (Gillingwater et al., 2007) and *in vivo* (Hagos et al., 2010), but for which parasite relapses have been reported after treatment of horses infected with *T. evansi* (Ravenborg, 1990; Wernery et al., 2001).

In this context, we report on the inability of Cymelarsan® to cure experimentally-infected horses with *T. equiperdum* in their CSF.

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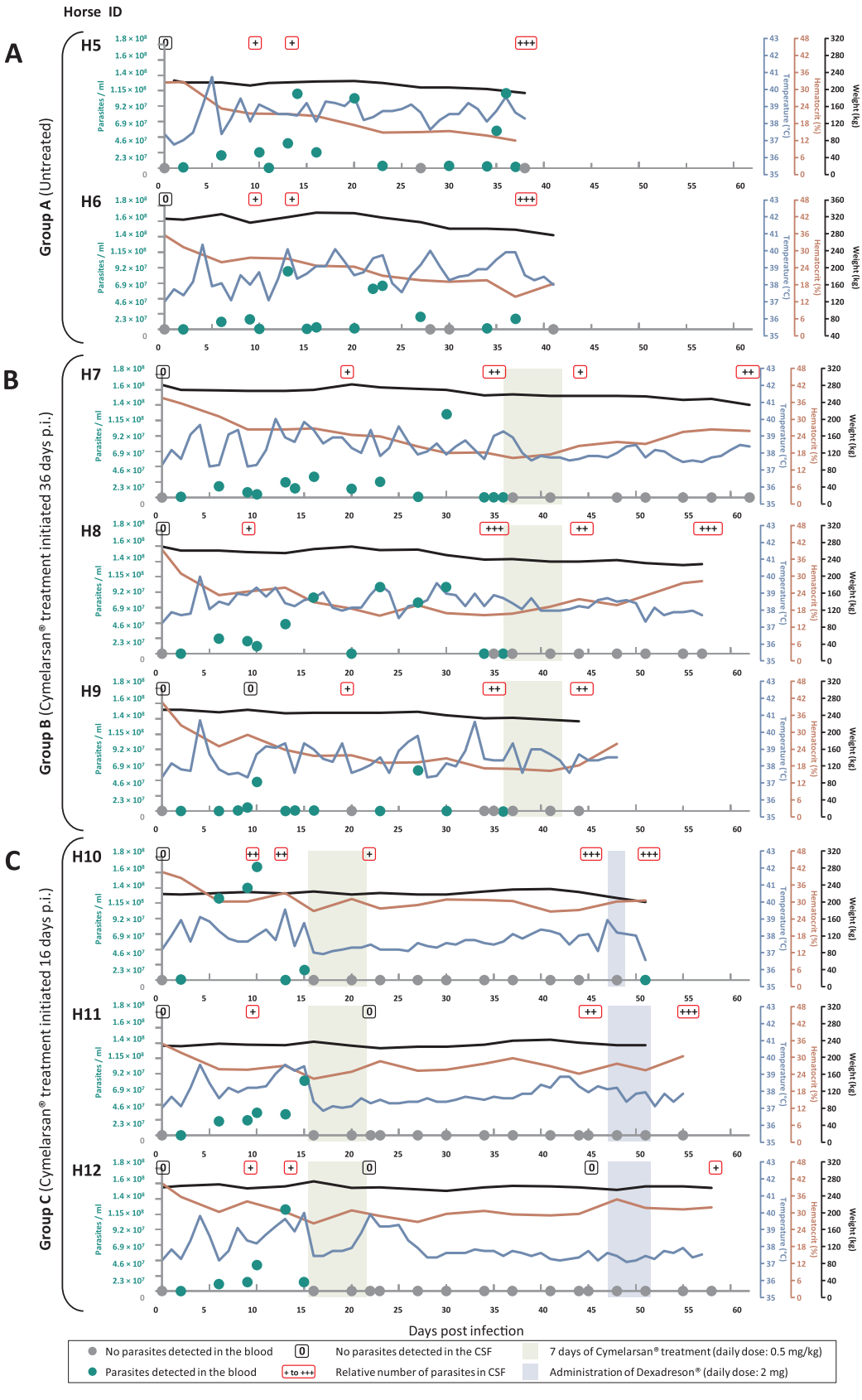

Fig. 1. Persistence of *T. equiperdum* parasites after Cymelarsan® treatment in the CSF of 8 experimentally-infected horses.

For the 8 horses infected (H5 to H12), the graphs show the number of parasites in the blood (green and grey dots), the rectal temperature (blue), the mean PCV (red), the weight (black), and the relative number of parasites observed in the CSF (boxed signs above the graphs). Two control horses remained untreated (A), whereas Cymelarsan® was administered intramuscularly at 0.5 mg/kg/day for 7 consecutive days (green boxes) for groups B and C, either 36 days p.i. for group B horses (B) or 16 days p.i. for group C horses (C). Group C horses were subjected to immunodepression on 47 days p.i. through the administration of 2 mg of Dexadreson® intramuscularly for 2 consecutive days (horse H10) or 5 consecutive days (horse H11 and H12) (blue boxes).

2. Materials and methods

2.1. Experimental infection of horses by T. equiperdum OVI

Eight Welsh pony mares (*Equus caballus*) of about 12 years old and seronegative for *T. equiperdum* were included in this study (Horses H5 to H12 from Hébert et al., 2018). All experiments were conducted in accordance with the guidelines of the directive 2010/63/EU of the European Parliament and of the Council, in the facilities of the Plate-Forme d'Infectiologie Expérimentale: PFIE, UE-1277, INRA Centre Val de Loire, Nouzilly, France. All experimental procedures were ethically approved by the Loire Valley ethical review board. Briefly, the experimental infections consisted for each animal of the intravenous injection of 5.10^4 living *T. equiperdum* OVI parasites propagated in rats (Hébert et al., 2018).

2.2. Blood sampling and recorded parameters

For each infected horse, blood samples from the jugular vein were collected in EDTA tubes (Venosafe™, Terumo® Europe) on day 0 and every 3 to 4 days after infection. The collected blood was used to monitor packed cell volume (PCV) and parasitemia by the matching method (Herbert and Lumsden, 1976). Rectal temperatures were recorded twice daily (around 9 a.m. and 4 p.m.) and weights twice a week (every 3 to 4 days).

2.3. Ultrasound-guided CSF sampling and parasite detection in CSF

To monitor the presence of parasites in the CSF of infected animals, CSF samples were collected from the subarachnoid space between the C1 and C2 vertebrae of standing horses by ultrasound-guided cervical centesis, according to the method described by Pease et al. (2012), with minor modifications (Hébert et al., 2018). To evaluate the presence of parasites, 4 ml of collected CSF was transferred into a transparent mini Anion Exchange Centrifugation Technique (mAECT) collector tube (IMT-INRB, Belgium, Democratic Republic of the Congo), centrifuged (10 min, 1600 $\times g$) and observed under a microscope (10×40) (Büscher et al., 2009). The relative number of parasites was reported with these corresponding scores: 0 (none), + (rare); + + (multiple) and + + + (numerous parasites). See supplementary data video online for an illustration of the CSF parasite loads.

2.4. Cymelarsan® treatment

The treatment consisted in administering 0.5 mg/kg/day of Cymelarsan® by the deep intramuscular route every day for 7 consecutive days, alternately in the middle third of the neck and in the rump. The 8 infected horses were randomly divided into three experimental groups. Horses H5 and H6 (group A) remained untreated during this study. The treatment was initiated on day 36 p.i. for horses H7, H8 and H9 (group B), and on day 16 p.i. for horses H10, H11 and H12 (group C).

2.5. Immunodepression induction

To increase the probability of detecting parasites in horses that relapse after treatment, immunodepression was induced on day 47 p.i. for group C horses. For 5 consecutive days, these horses were given 1 ml of dexamethasone sodium phosphate 2 mg/ml (Dexadreson®, Intervet INC, France) by a daily intramuscular injection into the rump. The immunodepression process was stopped for horse H10 after 2 days due to the deterioration of its general health.

3. Results

To evaluate the ability of Cymelarsan® to eliminate *T. equiperdum* OVI from the CSF of horses, we infected 8 Welsh pony mares (horse identification: H5 to H12) intravenously with 5.10^4 *T. equiperdum* OVI parasites. All the infected horses rapidly developed clinical signs of dourine, including parasitemia, anemia and fever, and the infestation of their CSFs was detected by ultrasound-guided cervical centesis (Fig. 1 and article Hébert et al., 2018 for details).

For H5 and H6 (group A), we let the infection develop without providing treatment, resulting in a worsening of the dourine symptoms, notably characterized by parasitemia, weight loss, anemia, fever, and a massive infestation of the CSF (Fig. 1A). Given the deterioration of their general health, group A horses were euthanized on 38 and 41 days p.i. respectively.

For horses H7, H8 and H9 (group B), we let the infection develop for 36 days. During this period, weight loss and increased CSF parasite load (Fig. 1B) were observed. The Cymelarsan® treatment (daily administration of 0.5 mg/kg of Cymelarsan® over 7 days) was initiated on day 36 p.i. After the end of the treatment, no parasites were detected in the blood of these horses, and we observed an improvement in the general health of the animals, reflected by normalization of hematocrit as well as by stabilization of the rectal temperature for all three horses. However, live parasites were observed in the CSF of the three horses (Fig. 1B) on day 43 p.i. Despite a potential decrease in parasite load observed in the CSF samples of horses H7 and H8 after treatment, the general health of these animals deteriorated and we had to euthanize horses H7, H8 and H9 on days 62, 57 and 48 p.i. respectively.

To evaluate the ability of Cymelarsan® to cure horses presenting a lower parasite load in their CSF, we initiated the Cymelarsan® treatment 16 days p.i. for group C horses (H10 to H12). After the end of the treatment, we observed an absence of parasites in the blood of these three horses, together with increased hematocrit and a stabilization of the rectal temperature (Fig. 1C). However, parasites were detected in the CSF of horses H10 and H11 on 22 and 45 days p.i. respectively, whereas no parasites were observed in the CSF of horse H12 up to 45 days p.i (*i.e.* 35 days after the end of the treatment). To increase the probability of a detectable parasite relapse in horse H12, we commenced the daily administration of an immunosuppressant (Dexadreson®) for 5 days from day 47 p.i. This treatment was also administered to horses H10 and H11 to evaluate its potential side effects (Fig. 1C). After 2 days, the immunosuppressive treatment was stopped for horse H10 because of a deterioration in its general health. Parasites were subsequently observed in its blood on day 51 p.i. Seven days after the end of the Dexadreson® treatment (day 58 p.i.), parasites were observed in the CSF of horse H12, therefore indicating the failure of Cymelarsan® treatment for group C horses.

4. Discussion

In the light of i) the report on the effectiveness of Cymelarsan® in *T. equiperdum* infected horses (Hagos et al., 2010) and ii) the recent report of an experimental model for assessing trypanocide efficacy on horses with *T. equiperdum* parasites in the CNS (Hébert et al., 2018), this study evaluates the efficacy of Cymelarsan® to cure animals suffering from a nervous form of dourine.

Inoculations of Welsh pony mares with 5.10^4 *T. equiperdum* OVI parasites resulted for all 8 ponies in infection characterized by the presence of parasites in blood and CSF, together with the development of typical clinical signs of dourine, including fever and anemia (Hébert et al., 2018 and Fig. 1). Even if the intravenous administration could have facilitated the host invasion, the rapid evolution of the disease, especially for the untreated group A horses, demonstrates the virulence of the *T. equiperdum* OVI strain and supports the hypothesis by Brun et al. (1998) that the adaptation of *T. equiperdum* strains to laboratory rodents could lead to the selection of virulent clones, which then cause acute infection in experimentally-infected animals.

For the horses that received the Cymelarsan® treatment, we observed an improvement in their general health, characterized by

normalization of the hematocrit and rectal temperature and clearance of the parasites from the blood (except for horse H10 51 days p.i.). These observations confirm the trypanocide action of Cymelarsan® and its proficiency to cure, at least apparently, horses suffering from trypanosomosis (Hagos et al., 2010). However, the fact that parasites were later detected in the CSF of all treated horses (only after the administration of an immunosuppressant for horse H12) revealed that this treatment is unable to eliminate the *T. equiperdum* OVI parasites located in the CSF or CNS of infected horses. These considerations could also explain the previously reported parasite relapses after Cymelarsan® treatment in horses experimentally infected by *T. evansi* or *T. equiperdum* (Ravenborg, 1990; Wernery et al., 2001). Another hypothesis to explain the treatment failure could be a development of parasite resistance to Cymelarsan® during the course of the study. This hypothesis seems however unlikely, in the light of a previous study that analyzed the resistance level of *T. evansi* isolated from the blood and CSF of relapsed horses after treatment with Cymelarsan® and which identified no indication of drug resistance (Ravenborg, 1990).

Unlike our study, Hagos et al., (2010) reported that Cymelarsan® was effective to cure horses infected by *T. equiperdum*. Two hypotheses could explain this divergence of outcome. The first is that during the Hagos et al., (2010) study, the Cymelarsan® treatment has been administrated to the infected horses while the parasites had not yet reached the CNS of its host, preventing the parasites to escape the treatment. The second hypothesis is that the *T. equiperdum* OVI strain is less sensitive to the Cymelarsan® than the *T. equiperdum* 834/940 Dodola strain used by Hagos et al. (2010). Indeed, it has been reported that the Cymelarsan® dose allowing to cure 5 of 5 infected mice was higher for *T. equiperdum* OVI (0.50 mg/kg) (Hagos et al., 2010) than for *T. equiperdum* 834/940 Dodola (0.25 mg/kg) (Habte et al., 2015). We can also note that *T. equiperdum* OVI has been reported as less sensitive *in vitro* to the Cymelarsan® than a panel of 19 other *T. equiperdum* and *T. evansi* strains, but the *T. equiperdum* 834/940 Dodola strain was not included in this study (Gillingwater et al., 2007).

Despite the apparent inability of Cymelarsan® to induce a permanent cure in our model, we observed a possible decrease in parasite load in the CSF of 5 horses (H7, H8, H10, H11, and H12) after Cymelarsan® treatment that could indicate the presence of low Cymelarsan® concentrations in the CSF of these equids. To test this hypothesis, it would be beneficial to proceed to a pharmacokinetic study of Cymelarsan® in the CSF of these animals. Given that it remains challenging to quantify arsenic compounds in biological samples (dos Santos Magalhães, 2014), the best alternative to assess the trypanocidal activity of Cymelarsan and/or its active metabolites in CSF consists in a bioassay that was previously used to determine levels of the trypanocidal drug melarsoprol in biological fluids (Onyango et al., 2000). Moreover, even if Cymelarsan® had some ability to reach the CNS of horses, it would remain difficult to increase the treatment dose due to the economic cost and potential adverse reactions induced by this arsenic compound (Hettlich et al., 2003).

5. Conclusion

In conclusion, our study shows that 6 horses presenting *T. equiperdum* OVI parasites in their CSFs were not permanently cured from the infection by the daily administration of 0.5 mg/kg of Cymelarsan® over 7 consecutive days. Therefore this outcome discourages the use of Cymelarsan® to manage a horse trypanosomosis outbreak, especially in a context when it is not technically feasible to proceed to CSF sampling in order to assess whether the parasites are already present in the CSF or CNS.

Ethics statement

The procedures involving horses received approval from the Ethics Committee of Val de Loire (France), DGRI agreement APAFIS#2015010908456425. Procedures involving rats received approval from the ANSES/ENVA/UPEC's Animal Ethics Committee under DGRI agreement APAFIS#4632-201632114333964. Animal studies were compliant with all the applicable provisions established by European directive 2010/63/UE. All the methods were performed by approved staff members in accordance with the relevant standard operating procedures approved by the abovementioned ethics committees. All the animals used in this study were handled in strict accordance with good clinical practices and all efforts were made to minimize suffering.

Conflict of interest statement

The authors declare no potential conflicts of interest with respect to the research, authorship, publication of this article and/or financial and personal relationships that could inappropriately influence this work.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetpar.2018.11.005.

References

Brun, R., Hecker, H., Lun, Z.R., 1998. *Trypanosoma evansi* and *T. equiperdum*: distribution, biology, treatment and phylogenetic relationship (a review). Vet. Parasitol. 79, 95–107.

Büscher, P., Mumba Ngoyi, D., Kaboré, J., Lejon, V., Robays, J., Jamonneau, V., Bebronne, N., Van der Veken, W., Biéler, S., 2009. Improved models of mini anion exchange centrifugation technique (mAECT) and modified single centrifugation (MSC) for sleeping sickness diagnosis and staging. PLoS Negl. Trop. Dis. 3 e471.

Carnes, J., Anupama, A., Balmer, O., Jackson, A., Lewis, M., Brown, R., Cestari, I., Desquesnes, M., Gendrin, C., Hertz-Fowler, C., Imamura, H., Ivens, A., Kořeny, L., Lai, D.H., MacLeod, A., McDermott, S.M., Merritt, C., Monnerat, S., Moon, W., Myler, P., Phan, I., Ramasamy, G., Sivam, D., Lun, Z.R., Lukeš, J., Stuart, K., Schnaufer, A., 2015. Genome and phylogenetic analyses of *Trypanosoma evansi* reveal extensive similarity to *T. Brucei* and multiple independent origins for dyskinetoplasty. PLoS Negl. Trop. Dis. 9 e3404.

dos Santos Magalhães, I.R., 2014. Current bioanalytical methods for pharmacokinetic studies of drugs used in neglected tropical diseases. Bioanalysis 6, 1711–1723.

Gillingwater, K., Büscher, P., Brun, R., 2007. Establishment of a panel of reference *Trypanosoma evansi* and *Trypanosoma equiperdum* strains for drug screening. Vet. Parasitol. 148, 114–121.

Habte, B., Bsrat, A., Ashenafi, H., Regassa, F., 2015. Efficacy of some trypanocidal drug against *Trypanosoma equiperdum* OVI in experimentally infected mice in Debre Zeit, Ethiopia. Eur. J. Biol. Sci. 7, 7–13.

Hagos, A., Goddeeris, B.M., Yilkal, K., Alemu, T., Fikru, R., Yacob, H.T., Feseha, G., Claes, F., 2010. Efficacy of Cymelarsan® and Diminasan® against *Trypanosoma equiperdum* infections in mice and horses. Vet. Parasitol. 171, 200–206.

Hébert, L., Guitton, E., Madeline, A., Géraud, T., Carnicer, D., Lakhdar, L., Pitel, P.H., Coste, M., Laloy, E., Giraudet, A., Zientara, S., Büscher, P., Laugier, C., Hans, A., Petry, S., Cauchard, J., 2018. Validation of a new experimental model for assessing drug efficacy against infection with *Trypanosoma equiperdum* in horses. Vet. Parasitol. 263, 27–33.

Herbert, W.J., Lumsden, W.H., 1976. *Trypanosoma brucei*: a rapid "matching" method for estimating the host's parasitemia. Exp. Parasitol. 40, 427–431.

Hettlich, B.F., Ryan, K., Bergman, R.L., Marks, S.L., Lewis, B.C., Bahr, A., Coates, J.R., Mansell, J., Barton, C.L., 2003. Neurologic complications after melarsomine dihydrochloride treatment for *Dirofilaria immitis* in three dogs. J. Am. Vet. Med. Assoc. 223, 1456–1461 1434.

Jennings, F.W., Gray, G.D., 1983. Relapsed parasitaemia following chemotherapy of

chronic *T. Brucei* infections in mice and its relation to cerebral trypanosomes. Contrib. Microbiol. Immunol. 7, 147–154.
OIE, 2013. Dourine. OIE - Terrestrial Animal Health Code (Chapter 12.3). World Organisation for Animal Health, Paris, pp. 1–2 Off. Int. Epiz. 2013.
Onyango, J.D., Burri, C., Brun, R., 2000. An automated biological assay to determine levels of the trypanocidal drug melarsoprol in biological fluids. Acta Trop. 74, 95–100.
Pease, A., Behan, A., Bohart, G., 2012. Ultrasound-guided cervical centesis to obtain cerebrospinal fluid in the standing horse. Vet. Radiol. Ultrasound 53, 92–95.
Ravenborg, T., 1990. Untersuchungen Zur Wirksamkeit Von Cymelarsan® Bei Der Experimentellen *Trypanosoma*-(Trypanozoon)-*evansi*- (Steel, 1885) Infektion Des Pferdes Auf Den Philippinen.
Scacchia, M., Camma, C., Di Francesco, G., Di Provvido, A., Giunta, R., Luciani, M., Marino, A.M., Pascucci, I., Caporale, V., 2011. A clinical case of dourine in an outbreak in Italy. Vet. Ital. 47 (473-475), 469–472.
Wernery, U., Zachariah, R., Mumford, J.A., Luckins, T., 2001. Preliminary evaluation of diagnostic tests using horses experimentally infected with Trypanosoma evansi. Vet. J. 161, 287–300.
Zablotskij, V.T., Georgiu, C., de Waal, T., Clausen, P.H., Claes, F., Touratier, L., 2003. The current challenges of dourine: difficulties in differentiating *Trypanosoma equiperdum* within the subgenus Trypanozoon. Rev. Sci. Technol. 22, 1087–1096.