horses is the Complement Fixation Test (CFT). However, studies have shown that CFT cannot differentiate between species of Trypanosoma as the CFT is not species specific [5]. The diagnostic significance of this test is therefore doubtful in countries where both *T. equiperdum* and *T. evansi* infections occur. Human sleeping sickness [6] caused by *T. gambiense* and surra in camels caused by *T. evansi* [2] can be diagnosed with *T. equiperdum* antigen. In collaboration with the Instituto G. Caporale, Teramo, Italy, we received 8 confirmed Dourine positive equine sera samples of different titres collected during the Italian outbreak in 2011 [4]. These 8 sera were further tested at CVRL using antigen preparations from 7 different *T. equiperdum* strains including ITMAS 170108 (OVI), ITMAS 070109 (Botat), ITMAS 261003B 943 (Ethiopian), ITMAS 220101 (American), ITMAS 211199A (French), ITMAS 241199B (German), ITMAS 290101 (Canadian), as well as *T. evansi* strain. *T. evansi* strain was isolated from a dromedary in Dubai [3]. The results of these investigations are summarized in Table 1. As seen from the Table, 8 sera reacted with different titres against different *T. equiperdum* strains as well as *T. evansi*. This proves that standardization of *T. equiperdum* antigen for use in CFT is an important issue.

Table 1
CFT results of 8 Dourine-positive Italian horse sera using *T. evansi* and different strains of *T. equiperdum* antigens

SAMPLE ID	CVRL CFT	CFT	CVRL CFT ** (with different *T. equiperdum* strains)						
	*T. evansi**	Teramo, Italy	Ethiopian Ag	Ovi Ag	French Ag	Canadian Ag	German Ag	BoTat Ag	American Ag
NAM 3	1:20++++	1:320++	1:160+	1:80++	1:40++	1:40+++	1:80++++	1:80+	1:80+
NAM 4	1:40++	1:640++	1:160++	1:80++	1:40++	1:80+	1:160+	1:80+	1:80+++
BATCH 0036	1:20+++	1:2560	1:40+	1:40+++	1:40+	1:40+	1:80+	1:20++	1:80++
BATCH 0037	1:20+	1:160	1:40++	1:40+++	1:20+	1:40+	1:80+	1:40+	1:40++
BATCH 0039	Negative	1:10	1:2++	1:5++	1:2+	1:2+	1:5++	1:2++	1:5++
BATCH 0038	1:160+	1:2560	1:640++	1:320++	1:320+	1:320+	1:320+	1:320+	1:640++
BATCH 0040	1:10++++	1:160	1:40+	1:40+	1:20+	1:10+++	1:80+	1:20++	1:40++
BATCH 0041	1:5++++	1:160	1:10+	1:5+++	1:2+++	1:5+++	1:10++	1:10+	1:5+++

*The optimum dilution of *T. evansi* antigen for CFT testing has been obtained by performing checker board with *T. evansi* positive serum

**The optimum dilution of different isolates of *T. equiperdum* antigen for CFT testing has been obtained by performing checker board with *T. equiperdum* positive serum.

References

[1] Gallo C., Vesco G., Campo F., Haddad N. & Awelmoula H. (1989). — Enquête zoo-sanitaire chez les chèvres et les dromadaires au sud de la Tunisie. Maghreb Vét., 4, 15-17.

[2] Kinne J., Wernery U. & Zachariah R. (2001). Surra in a guanaco (*Lama guanicoe*). JCPR, 8(2), 93-98.

[3] Pascucci I. & Cammà C. (2015). Updates and lessons from the 2011 outbreak in Italy, Workshop of the EU RL on Dourine, 2 October 2015, Paris-France.

[4] Pautrizel R., Lafaye A. & Duret J. (1959). — Diagnostic sérologique de la maladie du sommeil. 1. Amélioration de l'antigène préparé à partir de T. equiperdum. Bull. Soc. Pathol. Exot., 52, 318-331.

[5] Pautrizel R., Lafaye A., Duret J., Tribouley J. & Ripert C. (1962). — Etude comparative de l'évolution de différents anticorps élaborés au cours de trypanosomose expérimentale chez le lapin. Rev. Immunol. Fr., 26, 157-166.

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Preliminary investigations on the sequence heterogeneity of the 18S rRNA gene of *Theileria equi* and *Babesia caballi* strains collected from a horse population in Central Italy

A. Cersini [1], LE Bartolomé Del Pino [1,2], V. Antognetti [1], R. Lorenzetti [1], R. Nardini [1], G.L. Autorino [1], M.T. Scicluna [1]

[1] *Istituto Zooprofilattico Sperimentale Lazio e Toscana, Rome, Italy;* [2] *Complutense University of Madrid, Spain*

A molecular survey of equine piroplasms was conducted using samples collected from symptomatic and asymptomatic horses of Central Italy. Case definition for the acute form of piroplasmosis was temperature >38°C and at least one of the following signs, jaundice, anaemia and petechial haemorrhages, and a PCR positive result. Phylogenetic analysis was conducted on sequences of 78 blood samples collected in 2013/14, having either low Ct values in real-time PCRs (46 samples) for the parasites or a PCR pos/ELISA neg (VMRD, USA) result (32 samples). Sequencing was performed on the V4 hypervariable region of the 18S rRNA gene which was 390 bp for *B. caballi* and 430 for *T. equi* [1, 2]. Using BLAST, sequences were aligned with those deposited in GenBank for both piroplasms, having a minimum 98% query coverage and >97% homology. Genetic distance and homology confirmed that sequences of both parasites could be divided into the 3 groups, with a homology among the groups >97% [1, 2]. Group 1 included sequences homologous to first-ever reported piroplasms, group 2 to the "like genotypes" [1], and group 3 included those with equidistant homology for the two groups [2]. Among the 72 sequences identified as *T. equi*, 39 belonged to group 1, 24 to group 2, and 9 to group 3. Group 1 included 62% (24/39) of the sequences of symptomatic horses, while 96% (23/24) of T. equi like (group 2), were from asymptomatic horses. Studies report that sequences within group 1 and 2 are in all endemic areas, while in America only group 1 and 3 are present. To date, sequences belonging to Group 3 were never submitted for Europe and Asia. Of the 27 *T. equi* PCRpos/ELISAneg samples, 23 (85.1%) had Group 1 sequences with 19 of them from symptomatic horses. For *B. caballi*, 7 sequences were obtained, of which only 2 were from horses positive for ELISA, 4 belong to group 1, 1 to group 2, and 2 to group 3. No clinical significance was attributed to this parasite due to the limited number of sequences available. Sequences within the same groups and their wide geographic distribution suggest that the diversity could be independent from their origin and probably linked with the international movement of equidae. The present study is the first to report group 3 for both parasites

in Europe. Further studies are required to investigate the relatively low sensitivity of the ELISA and the possible correlation of the clinical evolution of the infection with the genetic group.

References

[1]. Nagore D, et al. (2004) Vet Parasitol;123:41-54.
[2]. Bhoora R, et al. (2009) Vet Parasitol;159:112-120.

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Anthelmintic resistance in Irish equine nematode populations

N. Elghryani, T. de Waal*
University College Dublin, School of Veterinary Medicine, Dublin, Ireland

The control of nematodes in grazing equids has been reliant on the intensive use of anthelmintic drugs for many decades. This has resulted in the development of resistance against the commonly used anthelmintic drugs in many countries. We undertook a survey to investigate the prevalence of anthelmintic resistance on 14 Thoroughbred- and two donkey- populations in Ireland. Anthelmintic efficacy against nematodes was determined by calculating the percentage reduction in the faecal egg count (FEC) between the group mean at Day 0 and Day 14 post-treatment. FECs were performed using the mini-FLOTAC technique. Animals were treated with either benzimidazoles, ivermectin or moxidectin drugs. Thresholds for appropriate efficacy were chosen at an arithmetic mean FECR of >95% for ivermectin/moxidectin and >90% for benzimidazole drugs. Based on these criteria, resistance to benzimidazoles was found in 1 group of adult mares (FECR =38%) and in in three groups of yearling horses (FECR range 0-86%). Ivermectin was effective on all populations with a FECR range between 95-100%. Reduced efficacy of moxidectin was observed in one yearling group (FECR = 67%) and in two adult mare groups (FECR range 89-92%). In addition FECs were also calculated at 2 week intervals for up to 16 weeks after anthelmintic drug administration to determine the egg reappearance period (ERP) for benzimidazoles, ivermectin and moxidectin. ERP was defined when the group arithmetic mean FEC exceeded 10% of the group arithmetic mean FEC at Day 0. The results indicated that ERP for moxidectin and ivermectin ranged from 28 − 56 days and 14 days for benzimidazoles. Overall the results from this study indicate that benzimidazole, ivermectin and moxidectin are still effective on the majority of farms. However, ERP results would suggest that these products are less effective compared to label claims as a shortened ERP is believed to be an early indicator of resistance. This highlights the need for more targeted approaches in the control of nematodes in Irish equine populations to slow down the impending development of multi-drug resistance parasite populations.