reversion to susceptibility are very slim. In the pre and post treatment stool cultures, all larvae obtained were classified as cyathostomes. Benzimidazoles resistance is widespread in the central area of Argentina [3] . In this context, the use of these drugs to control small strongyles could be currently inadvisable unless controls are carried out post treatment to establish efficacy thereof. Practitioners need to determine if anthelmintic resistance is present before advice control parasite programs. Presently, FECRT is the most appropriate method for these practices and should be used regularly to monitor the resistance status on horse farms.

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Isolation of *Sarcocystis neurona* from an opossum (*Didelphis albiventris*) in Argentina

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Sarcocystis neurona is an Apicomplexan parasite which affects a wide range of animal hosts. This protozoan is the main cause of equine protozoal myeloecephalitis (EPM) in Western Hemisphere horses. The parasite reproduces sexually in the intestine of definitive hosts (DH) and asexually in tissues of intermediate and aberrant hosts. The geographical distribution of *S. neurona* is related with the distribution its definitive hosts, the opossums *Didelphis virginiana* and *D. albiventris*. A recent serological study conducted in Argentinean horses using *S. neurona* antigen revealed an overall seroprevalence of 26.1%. However, the parasite has not been isolated in Argentina. Tissues from an opossum (*D. albiventris*) hunted by dogs in a farm from the central region of Buenos Aires province were collected. Horses raised in the farm showed a 50% (10/20) *S. neurona* seroprevalence. One seropositive horse developed neurological signs and evidenced clinical improvement after a 2 month treatment with Ponazuril. A complete necropsy of the opossum was conducted and the intestinal mucosal scraping was subjected to a parasitological study with sucrose solution. A high amount of *Sarcocystis* spp. oocysts/sporocysts were observed (Fig. 1). DNA was extracted from concentrated oocysts with a commercial kit (ZR Fecal DNA, Zymo Research). The sample was identified as *S. neurona* by specific PCR-restriction fragment length polymorphism (RFLP) and by sequencing of a fragment of the 18S rRNA gene. Approximately 5 x 10^5 oocysts were subjected to a pepsin-HCl digestion followed by a physical disruption. Released sporozoites were used to infect fresh BM cell cultures, maintained by 3 passages during 2 months and further preserved in liquid nitrogen. This study represents the first isolation of *S. neurona* in Argentina. Further studies will be conducted in order to identify antigen expression as well as to compare genetic characteristics between the isolated strain and reference strains.

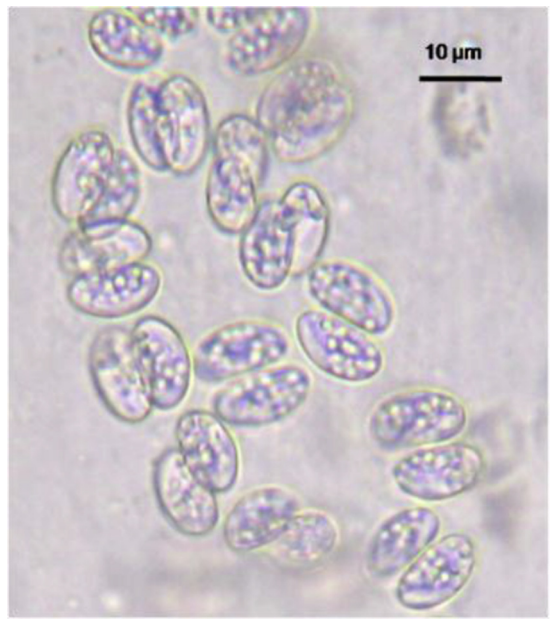

Figure 1. *Sarcocystis* spp. oocysts collected from opossum intestinal scrapping after sucrose flotation.

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Divergent CFT results of eight Dourine-positive horse sera using different *Trypanosoma equiperdum* and *T. evansi* antigens

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Dourine is caused by the protozoan parasite *Trypanosoma equiperdum* and is a serious, often chronic, venereally transmitted disease of horses and other equids. Once widespread, Dourine has been eradicated from many countries but may still be detected in horses in Asia, Africa, South America, Southern and Eastern Europe, Mexico and Russia. It was reported in June 2011 in Sicily and north of Naples, on the Italian mainland [1]. Dourine is an OIE listed notifiable disease. Laboratory diagnosis of Dourine is performed through serological tests such as CFT, IFAT and ELISA. The OIE recommended test for international trade of

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.

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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

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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