









process, and misidentification is not uncommon due to outdated identification keys or closely related species that are hard to distinguish. We are exploring the use of MALDI-TOF MS as a rapid, scalable, and reliable method of parasite identification for routine diagnostic purposes. Known tick species (Ixodes scapularis and Dermacentor variabilis) were acquired from a breeding facility at Oklahoma State University and used for optimization of the extraction protocols and for protein spectra generation. Molecular testing of the used samples is undergoing. A total of 301 field tick samples (Dermacentor and lxodes species) were collected as part of a bigger study and identified morphologically. These samples will be tested molecularly and with the MALDI TOF MS to assess the MALDI TOF MS as a method of identification of ticks. Protein spectra were successfully generated from ticks acquired from the breeding station and used to build MALDI-TOF MS spectra library. We are currently optimizing the protocol and generating MALDI TOF spectra from various specimens with both morphological and DNA barcoding molecular vouchers. The generated library was also challenged with spectra from other ectoparasites. MALDI TOF MS is a promising technology that has the potential to be adapted for use in parasitology diagnostic labs for parasite identification.

Haemonchus contortus isolates assessed for anthelmintic resistance using RESISTA-test©

César Cristiano Bassetto (Universidade Estadual Paulista, Brazil), Mariana Laura Elis Chocobar (Universidade Estadual Paulista, Brazil), Hornblenda Joaquinha Silva Bello (Embrapa Pecuária Sudeste, Brazil), Naiara Mirelly Marinho Silva (Universidade Estadual Paulis-

ta, Brazil), Alessandro Francisco Talamini do Amarante (Universidade Estadual Paulista, Brazil), Ana Carolina Souza Chagas (Embrapa Pecuária Sudeste, Brazil)

RESISTA-Test© is a larval development test to detect anthelmintic resistance for thiabendazole (TBZ), ivermectin (IVM), levamisole (LEV), and monepantel (MPT). Two isolates of Haemonchus contortus were assessed to confirm the anthelmintic status (Echevarria1991 - HcEc91, susceptible and Botucatu - HcBot, resistant) were kept in two donor lambs each. Eight lambs were artificially infected with each isolate and kept in separate paddocks (field lambs) for 160 days. To calculate the resistance factor (RF), faecal samples of the donor and field lambs of each isolate was examined using RE-SISTA-Test[©]. The results were analysed using logit dose-response by the Probit model. The RF was calculated as the LC50 values of the studied isolate divided by the respective susceptible isolate values. For the HcEc91 isolate in the field lambs, the RF values were TlA = 0.7, IVM= 0, LEV = 1.1 and MPT = 0.9. The RF values for the HcBot isolate in the donor lambs were TIA = 147, IVM = 176, LEV = 931, and MPT = 53 and for the field lambs the RF values were TIA = 141, IVM = 196, LEV = 822, and MPT = 34. The results confirmed with accuracy the anthelmintic resistance status of the two isolates since the RF values for the susceptible isolate were below 3 and the contrary was observed for the resistant isolate, with RF values above 3. Moreover, RF values for the donor and field lambs infected with *HcBot* isolate were guite similar for all chemical groups demonstrating that both isolates can be kept successfully in the field as long as lambs graze different paddocks. In conclusion, RESISTA-Test© is applicable in laboratory routines to ascertain the anthelmintic





resistance status of *H. contortus* without necessitating superfluous anthelmintic treatments such as those employed in faecal egg count reduction test (FECRT). This tool would be utilised in farm's parasite management initiatives, facilitating a more precise, and sustainable control of gastrointestinal nematodes.

Evaluation of a diagnostic method for *Theile-ria haneyi* using the flow cytometry-based hematology analyzer XN-31

Akihiro Ochi (Japan Racing Association, Japan), Reginaldo G. Bastos (United States Department of Agriculture, USA), Daiki Kishi (Japan Racing Association, Japan), Takanori Ueno (Japan Racing Association, Japan)

Equine piroplasmosis (EP) is a tick-borne disease caused by the intraerythrocytic protozoa Babesia caballi, and Theileria equi. A novel species, Theileria haneyi, has also been reported as a causative agent of EP, and the horses infected with *T. hanevi* have been identified in North America, South America, and Africa. EP is a reportable disease according to the World Organisation for Animal Health (WOAH), and infected horses are restricted from international movement. In Japan, diagnosis of EP is conventionally performed using microscopic, molecular, and serological methods. PCR-based diagnostic assays are the only available means to confirm *T. haneyi* infection in animal quarantine; however, it is time-consuming. Recently, we reported that the hematology analyzer XN-31 (Sysmex), originally developed for diagnosing human malaria, is efficient in detecting B. caballi and T. equi infections, as well asin differentiating between the two parasites. In this study, we evaluated the diagnostic potential of the XN-31 analyzer for *T. haneyi*. Evaluate

the use of XN-31 for diagnosis of *T. haneyi*. *T.* hanevi was in vitro cultured and measured by XN-31 (Sysmex, Kobe, Japan). The numbers of infected red blood cells (iRBC#) and ratio of infected RBCs (iRBC%) were counted by using XN-31 and by microscopic examination. XN-31 was assessed for limit of blank (LoB), limits of detection (LoD), quantitation (LoO), and linearity. XN-31 detected T. haneyi-infected RBCs (iR-BCs) in approximately 1 minute. To investigate the reliability of XN-31, iRBC% were compared between XN-31 analysis and microscopy examination. The correlation of iRBC% was high (R2 > 0.9). LoB was 0.7 cells/µl, and the LoD and LoQ were 6.8 cells/µl and 14.8 cells/µl. Linearity was good (R2 > 0.9). XN-31 detected T. hanevi-infected RBCs, reporting the infection ratio in approximately 1 minute. These findings indicate that XN-31 would be useful for EP diagnosis, especially for the monitoring of infected horses and for screening tests.

Antigens of *Neospora caninum* for the development of immunochromatographic and biosensor assays

Luciane Xavier Ferreira (USP, Brazil), Luciana Baroni (USP, Brazil), Luiz Miguel Pereira (University Center Estácio of Ribeirão Preto, Brazil), Amanda de Camargo Tardio (USP, Brazil), Júlia Mendonça Margatho (USP, Brazil), Juliana Cancino Bernardi (USP, Brazil), Ana Patrícia Yatsuda (USP, Brazil)

Neosporosis significantly impacts cattle production, resulting in annual losses of \$1.29 billion. There are no economically viable treatments or commercial vaccines available, making diagnosis key for animal management. This work aimed to characterize recombinant antigens as potential biorecognition molecules to develop