



## PA-P01

### Prevalence of *Rhabditis bovis* parasitic otitis in cattle farms Gyr in Córdoba, Colombia

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**Objetives:** Within the genus *Bos indicus*, the Indubrasil and Gyr breeds are more susceptible to *Rhabditis bovis* parasitic otitis. The Gyr breed due to its characteristics, in the Colombian Caribe has been selected for milk production. The objective was to determine the prevalence of *R. bovis* parasitic otitis in Gyr cattle farms in different livestock enterprises in Cordoba, Colombia.

**Materials and Methods:** A prospective descriptive study was implemented by convenience. Swabs of cerumen from ear canals of 136 cattle were collected in six cattle farms. The diagnostic criterion was the direct observation of the mass movement of cerumen and visualization of nematodes.

**Results:** The prevalence of parasitic otitis was 83,82%. Significant difference was found for the variables clinical otitis, otorrhea and odor, the sex variable was not analyzed.

**Conclusions:** Parasite diagnosis shows the existence of *R. bovis* as the cause of parasitic otitis in cattle in livestock farms in Cordoba (Colombia).

**Keywords:** Earwax, diagnosis, dual purpose, nematode.

## PA-P02

### Efficacy of a topical application of Cypermethrin, Clorpyrifos, Piperonil butoxide or Fluzuron on cattle naturally infested by *Rhipicephalus microplus* under grazing conditions of Brazil

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**Objectives:** This experiment aimed to compare the efficacy against *Rhipicephalus (Boophilus) microplus* in naturally infested cattle of two similar presentations of a topical formulation containing cypermethrin (5.0%), chlorpyrifos (7.0%), piperonil butoxide (5.0%) and fluzuron (2.5%), and a solution of Fluzuron (2.5%) alone, for pour-on application.

**Materials & Methods:** The study was designed as a comparative study with a negative control group. The research was carried out in a cattle farm at São José de Rio Pardo, São Paulo, Brazil (21°35 S, 46°53 W). Forty Simmental cattle were selected on the following criteria, 1) animals with at least 20 engorged females ticks in left side, parasite size ranged 4.5 < Ø < 8.0mm, counted from each animal on three con-

secutive days before treatment (days -3, -2 and -1), 2) live weight ranged between 350 to 450 Kgs, and 3) no history of application of acaricide within 60 days before enrolment. Animals were ear-tagged and then randomly allotted in one of the four experimental groups of ten animals, 1) G01 (cypermethrin, chlorpyrifos+piperonil butoxide and fluzuron. FORTIK 4, Virbac do Brasil, Brazil), 2) G02 (a product similar to G01. FLURON GOLD POUR ON, Ceva Saúde Animal Ltda, Brazil), 3) G03 (fluzuron. ACATAK, Elanco Brasil), and 4) G04, no treatment. A tick count was done daily on the restrained animals in all the body regions, including the head and ears, from 3 to 49 days post-treatment (DPT). Tick count is expressed as a geometric mean. Efficacy of treatment strategies is calculated from the relative risk for an animal to harbour more ticks than the control group at the same time point.

**Results:** Over the study period (DPT 3 to 49), the average *R. microplus* count significantly differed ( $p < 0.001$ ) between G01, G02, G03 on the one hand and G04 on the other hand (ANOVA, repeated measures). There was no statistically significant difference ( $P = 0.56$ ) in the mean tick counts between G01 and G02 during the experiment. Also, animals in G01 and G02 had mean tick counts significantly lower ( $P \leq 0.05$ ) than the animals from G03 on DPT3 and 7. Ultimately, there was no significant difference ( $p = 0.17$ ) between the three treated groups. Overall efficacy was  $\geq 95\%$  from DPT 14 to 21 for the three products, and  $\geq 80$ , from DPT 7 to 35, DPT 7 to 42 and DPT 14 to 42, for G01, G02 and G03, respectively. Then, the efficacy gradually declined and reached 56.9, 68.1 and 61.1 by DPT 49 for G01, G02 and G03, respectively (n.s.).

**Conclusion:** Based on the results from this experiment, the new combination of cypermethrin, chlorpyrifos, piperonil butoxide and fluzuron administered as a single pour-on treatment, showed residual protection against *Rhipicephalus (Boophilus) microplus* on naturally infested cattle managed under extensive conditions. This efficacy is similar to what is observed with a product formulated the same way. However, tick protection starts as soon as 3 DPT, which is not the case with a fluzuron 2.5% only-based product.

**Keywords:** Boophilus; fluzuron.

## PA-P03

### Prevalence and molecular characterization of *Cryptosporidium* and *Giardia* in pre-weaned native calves in the Republic of Korea

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**Objective:** *Cryptosporidium* spp. and *Giardia duodenalis* are protozoan parasites that cause diarrhea in humans and animals. Molecular data on *Cryptosporidium* spp. and *G. duodenalis* in calves in the Republic of Korea (ROK) is limited. This study aimed to investigate the prevalence of *Cryptosporidium* and *Giardia* in pre-weaned calves, analyze the association between these parasites and diarrhea, and identify the



zoonotic potential of *C. parvum* and *G. duodenalis* subtypes/assemblages.

**Materials and methods:** Between January and October 2018, 315 fecal samples were collected directly from the rectum of pre-weaned Korean native calves aged 1-60 days from 10 different farms in the Republic of Korea (ROK). Genomic DNA was extracted from 200 mg of each fecal sample using the QIAamp Fast DNA Stool Mini Kit. *C. parvum* was subtyped by targeting the 60-kDa glycoprotein (*gp60*) gene using nested PCR. *G. duodenalis* assemblage types were analyzed using the triose phosphate isomerase (*tpi*) gene,  $\beta$ -giardin (*bg*) gene, and glutamate dehydrogenase (*gdh*) gene. In this study, only samples showing a good sequencing result were considered to be positive for *C. parvum* and *G. duodenalis*.

**Results:** Overall prevalence of *Cryptosporidium* spp. and *G. duodenalis* was 4.4% ( $n=14$ ) and 12.7% ( $n=40$ ), respectively. Co-infection was not detected. All *Cryptosporidium*-positive samples were identified as *C. parvum* after sequence analysis of a small subunit rRNA fragment and further subtyped into zoonotic IIaA15G2R1 ( $n=13$ ) and IIaA18G3R1 ( $n=1$ ) by DNA sequencing of the 60-kDa glycoprotein gene. Based on  $\beta$ -giardin (*bg*) gene, *G. duodenalis*-positive samples belonged to assemblages E ( $n=36$ ) and A ( $n=4$ ), with the latter belonging to subtype A1, the zoonotic genotype. Six subtypes of assemblage E were identified at the *bg* locus: E1 ( $n=6$ ), E2 ( $n=3$ ), E3 ( $n=13$ ), E5 ( $n=1$ ), E8 ( $n=1$ ), and E11 ( $n=1$ ). The occurrence of *C. parvum* and *G. duodenalis* was not associated with diarrhea in pre-weaned Korean native calves. The prevalence of *C. parvum* is not related to calf age; in contrast, the prevalence of *G. duodenalis* was significantly higher in 41-50-day-old calves (odds ratio=9.90, 95% confidence interval: 2.37-41.34;  $P=0.001$ ) than in 1-10-day-old calves.

**Conclusions:** These findings suggest that calves may be an important source of zoonotic *C. parvum* and *G. duodenalis* infections. Because cryptosporidiosis and giardiasis prevention is important for maintaining good health of calves and humans, the risk of diseases caused by these parasites should be reduced by minimizing the infection pressure resulting from contamination of environment with *C. parvum* and *G. duodenalis* oocysts/cysts and by improving the immunity of calves.

**Keywords:** *Cryptosporidium*, *Giardia*, diarrhea, age, pre-weaned calves.

#### PA-P04

##### *Rhipicephalus microplus* resistance to acaricides in Brazil

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**Introduction:** *Rhipicephalus microplus* ticks have developed resistance to the majority of acaricides available on the

market in Brazil over time.

**Objective:** The objective of this study was to determine the resistance levels of *R. microplus* against commercially available acaricides at randomly selected commercial farms in Brazil. This study was a randomized, open label, resistance study.

**Materials and Methods:** For the study, 44 commercial farms were selected from seven states in Brazil for tick collection. The adult Immersion Test (AIT) was used to evaluate serial concentrations of fluzaron. Impregnated papers with alpha-cypermethrin were used as an indicator of resistance against synthetic pyrethroids, chlorfenvinphos as a general indicator of organophosphorus resistance, and amitraz as an indicator for formamidine resistance in the Larval Packet Test (LPT). Serial concentrations of moxidectin and fipronil were evaluated by the Larval Immersion Test (LIT). In order to perform paired tests, the Porto Alegre strain (POA), characterized as susceptible to all acaricides, was used as a reference strain. The criteria for classifying a sample as resistant with the LPT was effectiveness below 95% at the discriminating dose. For the LIT with moxidectin and fipronil, the criteria for the diagnosis of resistance was: a) susceptible - when the half maximal effective concentration (EC50) of the field isolate was not statistically different from the reference strain, b) incipient - when the EC50 of the field isolate was statistically different from the reference strain and resistance ratio (RR) < 2, and c) resistant - when the EC50 of the field isolate was statistically different from the POA strain and RR > 2. For fluzaron, resistance was considered when there was significant difference in index of fecundity between the field isolate and the susceptible reference strain. For each acaricide, the percentage of samples with resistance and the 95% confidence interval (CI95) were calculated.

**Results:** All evaluated samples were resistant to alpha-cypermethrin (CI95: 88.97 to 100), demonstrating the severe resistance status of *R. microplus* to synthetic pyrethroids in Brazil. The second active ingredient with the largest number of resistant samples was amitraz, with 87.5% of the farms indicating resistance of *R. microplus* (CI95: 71.93 to 95.03). Critical fipronil resistance status was also observed in 75.75% of the samples (CI95: 58.98 to 87.17). Sixty percent (CI95: 23.07 to 88.24) of the samples were resistant to fluzaron. However, only samples from five farms were evaluated for fluzaron resistance, because females had already started laying eggs before arriving at the laboratory or due to reduced number of viable ticks. Resistance to moxidectin and chlorfenvinphos was observed in 33.33% (CI95: 18.64 to 52.18) and 24.32% (CI95: 13.36 to 40.12) of the samples, respectively, indicating that moxidectin and organophosphorus products can still be used to control ticks in cattle on some farms in Brazil.

**Conclusion:** These results demonstrate that there is resistance to all active ingredients available on the Brazilian market for the control of *R. microplus*.

**Keywords:** *Rhipicephalus microplus*, resistance, acaricide.



## PA-P05

### The prevalence of the cattle parasite, *Babesia divergens*, in ticks collected from Irish farms and woodland

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**Objectives:** In the past bovine babesiosis (redwater fever) caused by the protozoan parasite, *Babesia divergens* was common along the River Shannon catchment and the western seaboard of Ireland representing a serious economic and animal welfare problem to Irish livestock farmers. While clinical cases have declined significantly over the last 30 years, the reasons for this decline are poorly understood. This study aimed to determine the current risk of bovine babesiosis by screening questing ticks in formerly endemic areas for the presence of *B. divergens*.

**Materials and methods:** Between 2018 and 2019, 736 *Ixodes ricinus* nymphs from 11 woodland sites and 448 *I. ricinus* nymphs collected from 14 farms (5 dairy, 9 beef) located in the midlands, the River Shannon catchment area and the west and southwest of the country, were screened for the presence of *B. divergens* using a TaqMan PCR protocol aimed at an 83bp fragment of the HSP70 gene. All ticks were collected by blanket dragging in accordance with ECDC tick survey guidelines. This involved 35 x 5 meter sweeps of a 1x1m piece of cotton fabric over vegetation and recording the number of ticks collected on the cotton sheet after each sweep.

**Results:** In total, 2.0% (15 out of 736) (95% confidence interval: 1.0% to 3.1%) of nymphs collected in woodland and 3.1% (14 out of 448) (95% CI 1.5% to 4.7%) of nymphs collected from livestock farms were found to be infected with *B. divergens*. All infected ticks were collected on dairy farms.

**Conclusions:** *B. divergens* infection levels in questing ticks were comparable to those reported from other European countries such as Poland (1.6%), Norway (0.9%) Italy (0.85%) and Switzerland (1.9%). The fact that there was no difference in prevalence rates between ticks collected from woodland compared to those collected from farms is probably a reflection of the fragmented nature of the Irish landscape.

**Keywords:** *Babesia divergens*, *Ixodes ricinus*.

## PA-P06

### A pilot study on the ectoparasiticides used by Irish farmers against lice and their efficacy

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**Objectives:** The objectives of this pilot study were to assess whether cattle lice are an issue on Irish beef cattle farms, to investigate what measures were used to control them and whether they were effective.

**Materials and methods:** During the winter of 2018/2019 and 2019/2020, 17 beef cattle farms were visited to interview the farmers about the louse control measures implemented on their farms. On each farm the level of louse infestation was assessed by examining an average of 35 animals. Lice were returned to the lab for identification to species level. Lice from 3 herds were tested for susceptibility to deltamethrin using bioassays according to FAO guidelines.

**Results:** The farmers surveyed used a range of ectoparasiticides to control lice including ivermectin, doramectin, deltamethrin, cypermethrin, diazinon and a product containing both ivermectin and closantel. 14 farm visits took place within 1 to 8 weeks post louse treatment while no treatment had yet been applied on the remaining 3 farms. At the time of the visit, 16 farms (94%) were positive for lice, with lice being detected on 10-95% of the animals sampled. Heavy infestations were recorded on 11.8% of farms. Lice were identified as *Bovicola bovis* (87.5% of farms) and *Linognathus vituli* (56.25% of farms). Bioassays on *B. bovis* lice collected from 3 farms indicated that the parasites were deltamethrin-resistant.

**Conclusions:** Cattle farmers in Ireland rely on a broad spectrum of ectoparasiticides to control lice control measures. Our survey provides preliminary data that indicate that on a small proportion of farms louse control measures failed to maintain infestation levels at low or medium levels. This study provides the first evidence of deltamethrin-resistance in chewing lice in Ireland.

**Keywords:** Lice, ectoparasiticides, resistance.



## PA-P07

**First report of anthelmintic efficacy in cattle received in Feedlot in Mexico**

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Currently, one of the worldwide challenges in parasite control is to generate efficient protocols that help to reduce the impact of neglected parasitic diseases in cattle. Due to the growing problem of anthelmintic resistance, and its expansion against most of the active chemical principles, they represent a threat to the viability of bovine production systems that directly affect the economy of local farms. The objectives of this study were to evaluate the anthelmintic efficacy in bovines received in fattening pens, as well as measure the daily weight gain for 99 days. The work was carried out in June 2019 at a feedlot in Tamuín, San Luis Potosí, Mexico (Praderas Huastecas SPR de RL). 180 male bovines (Swiss x Zebu) with 10-15 months of age and an average weight of 320 kilograms were used. Therefore, 24 hours after the reception of the cattle, stool samples were collected directly from the rectum of each animal. Through the Mc Master technique, 80 positive animals were diagnosed with gastrointestinal nematodes (300 eggs per gram of feces on average), which were randomly distributed to six experimental groups: Control group (n = 13) that remained without anthelmintic treatment; Group 2 treated with 1% ivermectin (IVM) (n = 13), with a dose of 0.2 mg / kg body weight subcutaneously; Group 3 treated with 1% Moxidectin (MOX) (n = 13), with a dose of 0.2 mg / kg body weight subcutaneously; Group 4 treated with IVM 2% and triclabendazole (TCB) 12% (n = 13), with an orally a dose of 0.2 mg / kg and 12 mg / kg body weight respectively; Group 5 treated with IVM 2%, TCB 12% and MOX 1% (n = 13), with an orally a dose of 0.2 mg / kg of IVM, 12 mg / kg of TCB and 0.2 mg / kg of MOX, subcutaneous route; Group 6 treated with 10% albendazole (ABZ) (n = 15), with an orally a dose of 5 mg / kg of live weight. At the start of treatment, there was no statistically significant difference in the elimination of eggs per gram of feces (P > 0.05). All animals were weighed before treatment and at the end of the experiment in order to calculate the daily weight gain (GDP). Anthelmintic efficacy (AH) was determined according to Abbot's formula (P > 0.05), with samples collected individually before and after treatment (day 0 and 14). The results indicate that the highest AH efficacy was obtained in the group treated with IVM / TCB / MOX with 100%, followed by the group treated with MOX with an efficiency of 98.5%, the group treated with ABZ 97.4%, the group treated with IVM 39.39% and finally, the group with the lowest efficacy (27.4%) was treated with IVM / TRC. Larval culture results indicated the presence of *Cooperia* spp. (61.4%), *Haemon-*

*chus* spp. (29.8%) and *Oesophagostomum* spp. (8.7%). The greatest weight gain was obtained in animals where Moxidectin with 265 and 211 grams per day was included in the control group, followed by the group treated with Albendazole 155gr and finally the animals treated with Ivermectin 133 and 94 grs. It is concluded that according to the efficacy of the molecules used in this study, the groups that presented the highest efficacy were those that included MOX with 100% and 98.5% efficiency.

**Keywords:** Fattening livestock, Feedlot parasites, Efficiency.

## PA-P08

**Preventive efficacy of toltrazuril (15mg / kg), against eimeriosis in experimentally infected calves**

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**Objective:** The present study evaluated the preventive efficacy of toltrazuril 5% (Baycox® - Bayer Animal Health), administered orally at a dose of 15mg / kg, against *Eimeria* spp. in experimentally infected calves.

**Material and Methods:** The study was conducted on a commercial farm located in the municipality of Jataí, Goiás, Brazil, from December 2019 to February 2020. Forty two calves (average age 30 days) which have not been treated with any anticoccidial drug since birth were recruited in the study and were kept in coccidia free clean environment before allocation to different groups. Feces from animals were examined for oocyst by McMaster technique on days -46, -45 and -42 pre challenge. Animals not shedding *Eimeria* oocysts were randomly allocated to 7 treatment groups (T01-T07) of 6 animals each. The animals of groups T01, T02, T03, T04, T05, T06 were treated with toltrazuril 5% (Baycox® - Bayer Animal Health) at 15 mg/ kg body weight on day -42, -35, -28, -21, -14, and -7, respectively. Animals of group T07, received saline solution at day -42. Each group was placed in a separate collective pen, with wood shavings litter, during the experimental period. On day 0 of the study, each animal in all groups was challenged with 100,000 sporulated oocysts of *Eimeria* spp from a mixed field isolate containing 68.2% *E. bovis*, 24.7% *E. zuernii*, 3.5% *E. alabamensis* and 3.5% of *E. ellipsoidalis*. The preventive efficacy of toltrazuril was calculated by comparing mean oocysts per gram (OoPG) of feces counts, from faecal samples collected at different days in different groups with control group.



**Results:** Two animals of Group T07 (control) exhibited diarrhea with the presence of blood on day 14 post challenge, and diarrhea with or without blood was not observed on subsequent days. The calves of other groups (T01-T06), did not show any clinical signs (diarrhea and / or dehydration) suggestive of infection by *Eimeria* spp. during the study period. The preventive efficacy was calculated as 67.8%, 47.9%, 85.4%, 86.2%, 97.7% and 100 %, for calves treated days -42, -35, -28, -21, -14 and -7, respectively at day 28 post challenge. On day 35 post challenge, the preventive efficacy reached 59.9%, 53.1%, 83.3%, 78.1%, 94.8% and 100% for day -42, -35, -28, -21, -14 and -7 treated animals respectively. The mean OoPG counts in the control animals were significantly higher ( $P \leq 0.05$ ) than those in the animals of the groups treated with toltrazuril on days -28, -21, -14 and -7.

**Conclusion:** The toltrazuril, administered orally at a dose of 15mg / kg, demonstrated preventive efficacy against *Eimeria* spp. The preventive efficacy was calculated as 100 % on day 21, 28 and 35 post challenge in animals which were treated 7 days before challenge. However, preventive efficacy on day 21, 28 and 35 post challenge in animals treated on 14 before challenge was 90.32, 97.70 and 94.79 % respectively whereas, for animals treated 21 days before challenge was 80.65, 86.21 and 78.13 % respectively.

**Keywords:** Bovine, diarrhea, oocysts.

#### PA-P09

### Efficacy of paromomycin against cryptosporidiosis, a field trial in two large Slovenian dairy herds

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**Objectives:** To test the efficacy of paromomycin on dairy farms with a known cryptosporidiosis problem.

**Material and Methods:** Thirty calves naturally infected with *Cryptosporidium* sp. from each of two large Slovenian farm were enrolled in the study (15 test calves treated with paromomycin and 15 control calves treated conventionally : fluid therapy, NSAID, antibiotic if indicated). Calves were separated from their mothers immediately after birth and raised in individual boxes until they moved to group boxes at about 3 weeks of age. In farm A, paromomycin treatment was started on day 4 after birth (in clinically healthy calves with positive HuveCheck® Crypto test) for 7 consecutive days; in farm B, treatment with paromomycin was started after cryptosporidiosis was confirmed in calves with diarrhoea. Dosing was in accordance with the manufacturer's instructions: 50 mg/kg bw per day (Huvepharma, Belgium). All calves were weighed at birth and at one month of age. In addition, oocysts were counted in faeces smears on days 11 and 16 using modified Ziehl

Neelsen staining.

**Results:** In farm A, we found statistically significantly better weight gain and fewer *Cryptosporidium* oocysts in faeces smears in the treated calves compared to the control calves. Although weight gain and oocyst count were on average better in the treated calves on farm B, the difference was not statistically significant. A better effect of paromomycin was observed when it was given before the onset of diarrhoea.

**Conclusion:** Since paromomycin is an antibiotic, prophylactic treatment cannot be recommended. However, metaphylactic use is beneficial during the calving peaks once cryptosporidiosis has been diagnosed.

**Keywords:** Cryptosporidiosis, paromomycin, calves.

#### PA-P10

### Latitudinal region affects the prevalence and hematological parameters of *Theileria orientalis*-infected dairy cattle in the Republic of Korea

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**Objectives:** The increasing distribution of tick-borne parasites and the re-emergence of tick-borne diseases as a result of climate change pose a global threat to dairy cattle. In this study, we evaluated the effects of a climatic factor, the latitudinal region, on the prevalence and hematological profile of dairy cows infected with *Theileria orientalis* in the Republic of Korea.

**Materials and methods:** Blood sampling was done on 365 non-grazing, clinically healthy, lactating Holstein-Friesian cows, from 27 dairy farms located in seven provinces in Korea, assigned based on latitude as northern, central, and southern regions. Samples were subjected to hematology analysis using an automated hematology analyzer and molecular detection of *T. orientalis* major piroplasm surface protein gene using polymerase chain reaction.

**Results:** The overall prevalence was 20.00%, from 70.37% of farms. The prevalence was significantly higher in the southern region (35.94%) and tends to become lower in the central (21.95%) and northern (12.92%) regions. The RBC and its extended parameters, HCT, HGB, MCV, and MCH were significantly downgraded among infected cows in the southern region.

**Conclusion:** This is the first nationwide study of *T. orientalis* prevalence in Korea, that assessed the difference between latitudinal regions among non-grazed, asymptomatic, lactating dairy cows, revealing *T. orientalis* is more prevalent in areas with warmer weather. With the influence of this climatic factor, more animals could have impaired health and productivity due to the downgraded blood profile incurred by subclinical *T. orientalis* infection.



**Keywords:** Theileria orientalis, Dairy cattle, Latitudinal region, Hematology.

#### PA-P11

### Concentration of antibodies against *Tritrichomonas foetus* surface antigen TF1.17 from sythetic mRNA-transfected cells

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**Objectives:** Antibodies against the TF1.17 surface antigen of *Tritrichomonas foetus* (TF) inhibit attachment to host cells and decrease parasite viability. Synthetic mRNA encoding these antibodies could be applied to the urogenital epithelium of bulls to prevent or treat trichomoniasis. Antibodies against TF1.17 have been produced by mRNA transfected preputial keratinocytes, but in concentrations too low to demonstrate biologically relevant effects. The objective was to develop an assay to concentrate functional expressed antibody against TF1.17.

**Materials & Methods:** Synthetic mRNAs for membrane anchored, bovine IgG antibodies against 2 epitopes of the TF1.17 antigen (TF1.15 and 1.17) were used to transfect (Messenger Max) A549 cells and bovine preputial keratinocytes (BPKs) for 24 hours. Phospholipase C (PLC; 1 U/mL) was used to cleave the antibodies from the cells and the supernatant was concentrated via centrifugal filtration. The concentration of bovine IgG was determined via ELISA (Abcam) and TF were treated with 1.0 µg/mL of TF1.15 or 1.17, and assayed for cytotoxicity via a commercially available kit (Promega CellTox Green).

**Results:** Bovine IgG yield from PLC treated A549 cells were determined to be 10.0 µg/mL and 5.5 µg/mL for TF1.15 and 1.17, respectively and were 10.0 µg/mL and 1.9 µg/mL for PLC treated BPKs. This confirmed that cells were expressing antibody and that transfection efficiency was higher for the TF1.15 construct in both cell types. Cytotoxicity evaluated via a fluorescent marker for membrane permeability revealed a 4-fold and 12-fold increase in relative fluorescent units from TF treated with TF1.15 and 1.17, respectively, as compared to untreated controls. While TF treated with both antibodies exhibited decreased viability, treatment with TF1.17 caused a larger decrease.

**Conclusion:** This method is feasible to concentrate expressed functional antibody for ongoing research to determine the efficacy of mRNA therapy to treat or prevent TF infection in bulls.

**Keywords:** mRNA, transfection, trichomoniasis, urogenital, cytotoxicity.

#### PA-P12

### Revised control methods for psoroptic mange in cattle in the face of emerging drug resistance

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**Objectives:** Current recommendations for the control of psoroptic mange in cattle were revised in the face of emerging acaricide resistance.

**Material and methods:** The published results of interviews with farmers, field efficacy studies and data simulation were used to revise control strategies for psoroptic mange in cattle.

**Results:** Treatment of psoroptic mange, caused by *Psoroptes ovis*, relies on the use of acaricides. Available chemical products in Europe are the macrocyclic lactones (MLs), flumethrin and, in certain countries, phoxim. Current recommendations for the control of psoroptic mange comprise of two treatments with a 7 – 10-day interval, with an acaricide at the recommended dose, of all affected and in-contact animals after removal of the crusts and shearing of the hair. Multiple studies have reported treatment failure after the use of MLs. This can be caused by suboptimal application of the treatment, e.g. underdosing, incorrect treatment interval or formulation and partial treatment of the affected group. In 2012, 54% of the farmers with problems with psoroptic mange in Flanders (Belgium) made at least one of these mistakes. Another possible cause of treatment failure is acaricide resistance. Results from an efficacy study from 2016-2019 in Flanders, Belgium detected treatment failure on 12 out of 16 farms with ML-resistance as most likely cause.

The presence of acaricide resistance imposed the need to re-evaluate current control recommendations. It will be essential to identify per farm the acaricides with an adequate efficacy, in order to achieve on-farm eradication of the disease. The mite count reduction test (MCRT) is the only available method to measure treatment efficacy in cattle and calculates the reduction in mite counts, based on pre- and post-treatment skin scrapings. A mean reduction in mite counts after correct treatment of ≥95% with a lower limit of the 95%-confidence interval of ≥90% is regarded as adequate drug efficacy by the authors. When the mean reduction and the lower limit are below these thresholds after correct treatment execution, drug efficacy is regarded as reduced. In other cases with a mean reduction or a lower limit of the 95%-confidence interval below their threshold, the efficacy is considered doubtful. Data simulation has identified the total number of skin scrapings per farm as the most important parameter for the diagnostic performance of the MCRT. For the detection of severely reduced drug efficacy (post-treatment mite count reduction <70%), a total of 36 skin scrapings per farm will provide reliable diagnostic performance. In order to detect early signs of reduced efficacy (70% - 86% efficacy), higher numbers of skin scrapings are needed.

Due to the limited number of available acaricides, it will be better for some farms to limit the presence and consequences of *P. ovis* infestations on their farm instead of aiming for disease eradication, as it is a realistic option that treatment



with any given acaricide will not result in a normal efficacy on certain farms. Even though, flumethrin resistance has not yet been identified in bovine *P. ovis*. However, *in vitro* resistance against pyrethroids has been reported in *Psoroptes cuniculi* in rabbits.

The recommendation to treat all in-contact animals can thus lead to further increased selection pressure towards more severe acaricide resistant mites. Therefore, it is recommended to evaluate per farm if it is necessary to treat all animals or only the clinically affected, based on the fraction of clinically affected animals and present on-farm possibilities to separate a group of animals. A group of clinically affected animals could be separated from the rest of the herd for treatment in order to sustain a susceptible refugee population within the animals without lesions.

**Conclusion:** The emergence of resistance has complicated the control of psoroptic mange. It is important for the farmers to know the efficacy of the used acaricides and use farm specific control strategies to prolong the longevity of these products on their farm.

**Keywords:** Psoroptic mange, control, resistance, diagnostics.

#### PA-P13

### Milk production and faecal egg counts in lactating dairy cattle

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**Objective:** Although research has indicated that anthelmintic use in lactating dairy cattle can result in increased milk production, much of this research has involved herds utilising housed dairy systems where cattle spend a portion of winter in stalls. Australian dairy systems are predominantly pasture-based. This may involve sustained exposure of cattle to gastrointestinal nematodes throughout life, allowing continued infestation but also ongoing immune system stimulation. Our aim was to assess the relationship between milk production and faecal egg counts (FEC) for individual cows in early lactation to determine whether there is a production deficit in animals with a higher FEC.

**Materials and Methods:** We measured the FEC of recently calved cows (less than 30 days in milk) in 11 commercial, pasture based dairy farms in south-west Victoria, Australia. Fifteen primiparous and 15 multiparous animals were selected based on age (multiparous; 3-4 animals from each age up to 6 years of age) and calving date. FEC was measured for individual animals at a sensitivity of 2.5 eggs per gram of faeces (epg). Farm data such as recent anthelmintic use, management, and cow body condition score (BCS) at sampling was recorded. Six farms used daily milk meters to analyse milk production whilst another six obtained regular herd tests (individual cow milk production assessments) through a contractor. These data were extrapolated to produce 100d production

figures. Data analysis was then undertaken using the Jamovi statistical package.

**Results:** Preliminary data from the herds with daily milk monitors (six farms) indicates a milk production deficit of 2.85L per day for the first 100 days in cows with FEC greater than 2.5 epg. This relationship is not consistent once farm or origin is accounted for. Analysis of the production data from the remaining six farms is required before an explanation of the data can be attempted.

**Conclusion:** FEC at a sensitivity of 2.5 epg may be an appropriate measurement of worm burden in pastoral dairy systems, and in some instances may be correlated with changes in milk production. However, further investigation of this data set is required before application of FEC can be endorsed as an appropriate tool for decision making.

**Keywords:** Milk production, FEC, Dairy, pasture-based, Ostertagi.

#### PA-P14

### Evaluation of condemned livers from major cattle due to lesions compatible with parasites in Spain

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**Objectives:** Parasitic lesions are one of the main causes of bovine liver condemnation notified at slaughterhouses by the Official Veterinary Service (OVS) in Spain. The objective of this study was to characterize condemned livers from major cattle due to the presence of lesions compatible with parasitic infection in a slaughterhouse in southern Spain, to confirm their aetiology.

**Material and Methods:** To carry out this study, a total of 29 livers of major cattle from 16 different origins were selected (2 from dairy and the others from beef) at the slaughterhouse. Selection criteria was the presence of gross lesions representative of the main lesions observed during each sampling day that originated the condemnation of the organ due to lesions compatible with parasites by OVS. Whole livers were transferred to the laboratory where the following analyses were performed:

(1) Macroscopic evaluation of the surface and serial sections of the parenchyma with a distance of approximately 3 cm in search of internal lesions.

(2) Microbiological culture in blood agar supplemented with 5% defibrinated and sterile sheep blood incubated at 37°C in aerobiosis and anaerobiosis for 24 to 72 hours. When granulomatous content was observed, a duplex PCR against *Mycobacterium tuberculosis* complex and *Mycobacterium avium* complex was also performed (n=4).



(3) Evaluation of the content of the lesion under a stereoscopic magnifying glass and microscope to assess the presence of specific structures as protoscoleces or parasitic membranes.

(4) When necessary three different real time PCR (qPCR) assays were used in order to identify Cestode spp. on these samples (n=13), but also for *Echinococcus granulosus* (hydatidosis) and *Taenia hydatigena/Cysticercus tenuicollis* (n=12).

(5) Microscopic evaluation of liver samples containing parasitic-like lesion or any other type of lesion previously fixed in 10% neutral buffered formaldehyde and routinely processed to carry out the histopathological analysis. A record was performed identifying cystic lesions, hepatocyte degeneration, inflammation, fibrosis and any other lesion of interest.

**Results:** The most frequent causes of condemnation were hydatidosis (hydatid cysts formed by the metacestodes of *Echinococcus granulosus*), with or without hepatic fibrosis or abscesses (51.7%) and chronic nonspecific parasitic processes in which it was not possible to confirm the causal agent (17.2%). Only 2 out of 15 (13.3%) examined hydatid cysts produced protoscoleces and could be considered fertile cysts. Other diagnosed processes were fibrosis with or without liver degeneration (6.9%), abscesses, lithiasis, non-specific chronic hepatitis and hepatodystrophy (3.4% respectively). Regarding the nonspecific fibrosis diagnosed, the high frequency of parasitic lesions could indicate that it could be associated with the migration of parasitic forms. Although visceral cysticercosis due to *C. tenuicollis* is the most frequent parasitic disease in other livestock species evaluated in the same geographical area (sheep and pigs, data not shown) all samples analysed in this study were qPCR negative to this pathogen. Abscesses diagnosed in our study (10.3% analysed samples) were originated by anaerobic filaments, mainly *Fusobacterium necrophorum* (necrobacillosis).

**Conclusions:** Hydatidosis was the most frequent cause of major cattle liver condemnation in our study. As in previous reports, most cattle livers carried infertile cysts that do not produce protoscoleces and are unable to continue the life cycle of the parasite. This result highlights the importance of applying molecular techniques (qPCR) to confirm the aetiology of these lesions, especially when there are no preserved structures in chronic lesions or infertile cyst. In addition to dogs, other canids such as foxes, frequent in the geographical area of study, are also definitive hosts of *E. granulosus*. Therefore, in order to reduce parasitic lesions in the slaughterhouse, the presence of definitive hosts must be equally controlled through biosecurity measures that prevent the coexistence of these animals and domestic livestock as well as the onset of periodic antiparasitic treatment in domestic dogs.

**Keywords:** Hydatidosis, cattle, liver, parasites.

#### PA-P15

#### *In vitro* anthelmintic activity of Praziquantel and Nitazoxanide for controlling *Eurytrema coelomaticum*

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**Objectives:** The objective of the present study was to verify, separately, the *in vitro* anthelmintic activity of praziquantel (PZQ) and nitazoxanide (NTZ) in the *E. coelomaticum* adult parasite and the histopathological lesions induced by these drugs. Until now, anthelmintic drugs capable of controlling this parasitosis are unknown, since despite the proven effect of praziquantel, there are no commercial formulations with this pharmacological basis for ruminants. The affected animal usually has subclinical pancreatic disease, but the damage caused to health and animal production are subdued. Therefore, the evaluation of new molecules in order to control this globally disseminated parasite with high prevalence in the southern region of Brazil is justified.

**Materials and Methods:** *E. coelomaticum* specimens were obtained from the pancreases of naturally infected cattle, collected from animals slaughtered in the city of Concórdia-SC, Brazil. A total of 180 parasites of uniform size and weight were used, distributed in tissue culture plates with six wells each and ten helminths per well, forming three groups: Group I negative control group (n = 60); Group II positive control group (n = 60) treatment with 80µg/mL of PZQ, and Group III treated group (n=60) treatment with 200µM of NTZ. The drugs were diluted in dimethylsulfoxide (DMSO) and separately added to the culture medium at a maximum concentration of 0.02% (v/v). In the negative control group, sheep serum and DMSO were used at the maximum concentration of the treatment groups, but without the drugs. Parasites in culture medium were kept in incubators at 37°C with 5% of CO<sub>2</sub>, being evaluated for motility after 3, 12, and 15 hours of incubation in a Stereomicroscope with a 20x magnification, using the following criteria: 3 (normal movement), 2 (slow movement), 1 (very slow movement), and 0 (no movement, dead). After the incubation period of 15 hours, the parasites were placed in a 10% buffered formalin solution for a minimum of 24 hours and sent for histopathological analysis. The parasites were dehydrated in increasing alcohol solutions, added in paraffin blocks, microtomed (3µm), and stained by the hematoxylin and eosin (H&E) staining method. Under an optical microscope, histopathological alterations in the organs of *E. coelomaticum* were analyzed comparatively between the negative control group, NTZ, and PZQ in 100x and 400x increase. The motility degrees between the groups were analyzed using the SAS program 9.3, submitted to the chi-square analysis (PROC





FREQ). Significant statistical differences were considered when  $P < 0.05$ .

**Results:** After 12 hours of incubation all parasites of the NTZ and PZQ groups were motionless or dead, while in the negative control group, 82% (5/60) presented normal motility after 15 hours of incubation ( $p < 0.001$ ). Histopathological examination showed severe damage in the vitellogenic gland, intestine, parenchyma, integument, and testicle in both treated and positive control groups. The vitellogenic gland showed disorganization of the acinus, necrosis, and a presence of glandular secretory content dispersed throughout the parenchyma. In the intestine, flattening of villi with a decrease in basal cells that line the organ was observed. The integument showed decreased eosinophilia, basal lamina discontinuity, and subtegumentary vacuolization.

**Conclusions:** It was concluded that PZQ and NTZ showed *in vitro* anthelmintic action against the parasite, as they caused significant lesions in the evaluated organs and reduced the parasite's motility. The NTZ may be an alternative drug to the use of PZQ in the controlling of *Eurytrema coelomaticum*.

**Keywords:** Euritrematosis, histopathology, nitazoxanide, pancreas, praziquantel.

#### PA-P16

### **Eurytrema coelomaticum infection: correlation between parasite burden and impairment of pancreatic exocrine enzyme secretion**

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**Objectives:** The objective of the present study was to determine if there is an impairment of exocrine pancreatic function and correlate it with parasite burden in *Eurytrema coelomaticum* infection. Despite some authors pointing out the disease as a cause of death, it is almost a consensus that the disease does not produce clinical signs, but is a silent disease that causes losses in milk and meat production. In addition, the infection of humans with this pancreatic trematode is also possible. Therefore, an accurate clinical and laboratory diagnosis is important for the best elucidation of the disease, since the definitive diagnosis is currently only reached with the necropsy of the animal or post-mortem examinations in slaughterhouses.

**Materials & Methods:** Pancreases, blood, and fecal samples were collected from 119 bovines at an abattoir. Stool samples were subjected to the gelatin and x-ray film digestion tests (to detect the presence of trypsin in feces). Using blood

samples, the following biochemical tests were performed: amylase, lipase, glucose, fructosamine, cholesterol, triglycerides, total protein, albumin, and globulins. The renal function of the animals (urea and creatinine) was also evaluated, as renal diseases can increase the enzymes amylase and lipase due to deficiency in excretion, thus, interfering with the results of the exocrine pancreatic function by causing a false positive for pancreatic lesions. The pancreases were placed in individual trays, isolated, and washed. All the water used in the washing was sieved through nylon sieves to separate the trematodes. Subsequently, the pancreatic ducts were opened with the aid of a scalpel, forceps, and surgical scissors, and the trematodes were removed using surgical tweezers and counted. The data were analyzed using the SAS Software. In order to analyze the relationship between the number of *Eurytrema* sp. with blood variables, the data were evaluated using Spearman's correlation analysis (PROC CORR). Logistic regression analysis (PROC LOGISTIC) and the odds ratio calculation were carried out to identify the odds ratio of the low and high parasitemia groups to present changes in blood variables concerning the non-parasitized group.

**Results:** Initially, the animals were classified as parasitized or non-parasitized by *Eurytrema coelomaticum*, with 71 positive and 48 negative cases, resulting in a frequency of 59.66%. The mean number of specimens per infected pancreas was 628 parasites, ranging from 6 to 3,829. Cattle with a high parasitic load presented a higher incidence of negative tests in both gelatin digestion and x-ray film digestion tests ( $P < 0.001$ ) when compared to non-parasitized animals and those with a low parasitic load. Changes in those tests only occurred if the parasitemia was moderate or severe. The activity of the amylase and lipase enzymes was significantly higher in animals with low parasitemia ( $P < 0.05$ ), compared to non-parasitized animals and with a high parasitic burden.

**Conclusions:** There was an absence of pancreatic digestive enzymes in the feces of highly or moderately parasitized animals. In addition, elevated serum levels of lipase and amylase were seen in animals with low burden, which also indicated the disease is compromising pancreatic exocrine functions.

**Keywords:** Amylase, Euritrematosis, lipase, pancreatic insufficiency, pancreatitis.

#### PA-P18

### **Cryptosporidiosis in calves of Croatian dairy farms: A neglected condition without reason**

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**Introduction:** Apicomplexan parasite *Cryptosporidium parvum* is one of the most important pathogens of young ruminants, particular newborn calves. Infected animals may suffer from acute watery diarrhoea, inappetence, lethargy leading to dehydration and in severe cases with lethal outcome. Even though cryptosporidiosis is widespread and has been reported



as a significant cause of enteritis worldwide, little is known on the occurrence in South-Eastern Europe, including Croatia.

**Materials & Method:** In the current study, 98 faecal samples were collected from individual calves under 21 days of age with diarrhoea from 10 farms. Faecal samples were examined using direct immunofluorescence test (MERIFLUOR Cryptosporidium/Giardia, Meridian Bioscience Inc., USA) and commercial point-of-care immunochromatographic test kit (SPEED V DIAR 4, Virbac, France). Samples were considered positive when the outcome was positive at one of the two tests at least.

**Results:** The infection with *Cryptosporidium* sp. was detected in 8 out of 10 (80%) farms surveyed with an overall prevalence of 38% (27/98) infected calves. In the positive farms, prevalence varied from 11% up to 50%. Interestingly, the immunochromatographic test showed higher sensitivity (27/27; 100%) compared to direct immunofluorescence assay (25/27; 92.6%).

**Conclusions:** Result of the current study has clearly shown that *Cryptosporidium* sp. is an essential and highly prevalent causative agent of neonatal diarrhoea in dairy calves and should consider more attention from both veterinarians and farmers. Furthermore, results from the present study showed that the immunochromatographic test performed slightly better than the direct immunofluorescence test, and could be used for fast detection of cryptosporidiosis on farms.

**Keywords:** Cryptosporidium, calf, Croatia.

#### PA-P19

### Seroprevalence and risk factors associated to *Neospora caninum* and *Toxoplasma gondii* in breeding sheep in the west of Mexico

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The objective of this study was to determinate the seroprevalence and risk factors associated to *Neospora caninum* and *Toxoplasma gondii* in breeding sheep in the west of Mexico. They were collected 184 blood samples of six municipalities in the state of Jalisco, Mexico. The taking of samples was made through venipuncture of the jugular vein by using tubes without anticoagulant, after this they were taken to the laboratory where they were centrifuged for the collection of serum, placing it in tubes of 1.5 milliliters and stored -20°C until their use. At the moment of realizing the visit of the collection of samples it was made a survey to the producers with the finality of identifying the risk factors. For the diagnose of *Neospora Caninum* and *Toxoplasma gondii* it was used the commercial kit of indirect ELISA following the recommendations of the manufacturer. The results showed a general seroprevalence of 16.32% (28/184) for *Neospora caninum* and 66.16% (114/184) for *Toxoplasma gondii*. Among the risk factors that

were identified they stand out the presence of the definitive hosts (dogs and cats) in the production unit, being proper or foreign, the direct contact with the definitive hosts, the manage of aborts as part of the personnel, and the closeness to the urban core. As a conclusion the, animals present a high seroprevalence with the two evaluated parasites if we compare it to other studies made where it was applied the same diagnose technique, this indicating that sheep are exposed to the infection of *Neospora caninum* and *Toxoplasma gondii*, meaning that it can cause lose in the production related to reproductive problems and abortions.

**Keywords:** Seroprevalence, Neospora, Toxoplasma, Sheep.

#### PA-P20

### Efficacy of two novel oral formulations of Flubendazole and Flubendazole/Triclabendazole combination in lambs in Uruguay

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**Objectives:** The objective of this study was to evaluate the efficacy of two novel oral formulations of flubendazole against *Haemonchus contortus* and *Fasciola hepatica* in lambs: flubendazole alone and a flubendazole/triclabendazole combination.

**Materials and Methods:** Study 1: Thirty lambs (24,3 ± 4.8 kg of BW) were artificially-infested orally with 2,500 3 status of *Haemonchus contortus* larvae, 37 days prior to initiating the Controlled Faecal Egg Count Reduction (FECR) test. The animals were allocated into three groups: FBZ group: treated with Flubendazole at 10 mg/kg (Flubenzin Suspension®, Calier, Uruguay); FBZ/TCZ group: treated with a combination of Flubendazole [10mg/kg] and Triclabendazole [10 mg/kg] (Flubenzin Forte®, Calier, Uruguay) and CTR group (untreated group). Individual faecal egg counts were performed prior (-10 days) and post (14 days) treatment.

Study 2: Eighteen lambs (30,1 ± 4.9 kg of BW) were artificially-infested with 200 metacercaria of *Fasciola hepatica*. The animals were allocated into two groups: FBZ/TCZ group: treated with a combination of Flubendazole [10mg/kg] and Triclabendazole [10 mg/kg] (Flubenzin Forte®, Calier, Uruguay) and CTR group (untreated group). All animals were necropsied at day 84 post treatment. Efficacy was assessed by the reduction of the number of parasites in the treated group in relation to the control group.

**Results:** Results in the study 1 showed a FECR of 95% [CI 94%-96%] and 92% [CI 91%-94%] in FBZ and FBZ/TCZ groups, respectively. The FECR in CRT group was 0% [CI 0 – 4%].

Results in study 2 showed a mean [range] of 39 [20-48] flukes in CTR group, whereas the fluke burden in the FBZ/TCZ



group was 0, resulting in 100% efficacy.

**Conclusion:** These results confirm the efficacy and sensitivity of two novel formulations of Flubendazole when used in lambs to control *Haemonchus contortus* and *Fasciola hepatica*. This adds two more alternatives to the parasite control in sheep farms.

**Keywords:** *Fasciola*, *Haemonchus*, Flubendazole; Triclabendazole; sheep.

## PA-P22

### ***Trypanosoma* sp. infection in cattle in Argentina. Distribution and characterization of diagnosed cases**

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*Trypanosoma* spp. are protozoan hemoparasites that cause different clinical manifestations in various animal species, including man. The animals can be carriers or develop acute to chronic clinical signs. These may be of different severity, even with an important economic-social impact. In America, the main agent is *T. vivax*, of mechanical transmission by hematophagous vectors such as *Stomoxys* sp. and *Tabanus* sp. It is also spread by iatrogenia. In Argentina, *T. vivax* was diagnosed for the first time by Monzón in Formosa in 2006. Since 2016, we have observed several clinical presentations in different dairy and beef herds from central Argentina, located in the province of Santa Fe, Córdoba and Santiago del Estero. The most frequent signs were anemia, weight loss, lower production, abortions, edema, diarrhea, sudden deaths. In some cases, there were misdiagnosis, confusing the disease with anemic diseases (anaplasmosis, babesiosis), abortigenic diseases (fetopathies such as leptospirosis), digestive processes (mycotoxicosis). The values of packed-cell volume and proteinemia were variable, the liver profile (GGT and GOT) was altered, and the Woo technique positive in some cases. The main lesions correspond to a syndrome of anemia and a generalized lymphadenomegaly. Some cases with hemoperitoneum, without rupture of the spleen or large blood vessels. Microscopically, lymphadenitis and anemia lesions were observed, such as centrilobular necrosis, erythrophagocytosis. The severity of the cases was diverse, influenced by the productive system (beef or dairy), level of production (advanced gestation, peripartum, high production), stressful situations (sudden death in caloric stress). The severity of the cases was diverse, influenced by the productive system (beef versus milk), level of production (advanced gestation, peripartum, high production), stressful situations (sudden death in caloric stress). Some cases were closely related to

massive vaccinations, and in very few there was a co-infection with *Anaplasma marginale*. In some of them it was possible to have a PCR molecular analysis corresponding to *T. vivax*. The treatment established had a positive effect with few recurrences. There were untreated parasitized animals with no signology (asymptomatic). In some of these asymptomatic herds, the reproductive indexes were lower than in previous years. It is essential to identify the hematophagous vectors present, to know their ecology, distribution and behavior, in order to prevent and control the diseases transmitted by them, and to determine how weather conditions may influence the dynamics of infection. The productive conditions of domestic animals must be considered in order to improve their immunological status. In addition, the trade and movement of animals between regions must be known, understood and analyzed. Applying the concepts of spatial epidemiology and medical geography is essential to prevent the consequences of this and other diseases.

**Keywords:** *Trypanosoma*, cattle, Argentina.

## PA-P26

### **Prevalence associated with natural infection of *Sarcocystis* spp. in fattening sheep in the state of Jalisco, Mexico**

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The objective of the present investigation was to determine the prevalence of *Sarcocystis* spp. in fattening sheep in the state of Jalisco, Mexico. 132 tissue samples were collected from slaughtered sheep in the municipality of Yahualica de Gonzalez Gallo Jalisco. The animals came from farms located in the municipalities of Cañadas de Obregón, Cuquío, Mexicacán and Yahualica de González Gallo. The collection of samples was carried out after the sacrifice, taking approximately 15 centimeters of the esophagus and 100 grams of the heart, which were placed in a plastic bag. The samples were transferred to the zoology laboratory of the Autonomous University of Guadalajara, where they were stored at -20°C until analysis. Prior to analysis, the excess fat in the tissues was removed to facilitate handling and to have a better view. The diagnosis was made by direct observation of the tissues, with the help of a stereoscopic microscope, dissection forceps and a scalpel. They were considered positive when observing white, round, or oval cysts on the tissue, with measurements ranging between 1.5 x 1.0 x 1.0 cm, with a white capsule and translucent gelatinous material inside. An overall prevalence of 2.27% (10/132; 95% CI 2.41 – 2.13) was identified. When performing the analysis according to the type of tissue, it was observed that the prevalence for heart was 10.61% (7/66; 95% CI 10.74 - 10.46) while in the esophagus the prevalence was 4.55% (3/66; 95% CI 4.68 – 4.40). A chi square test was performed to determine the association between the presence of cysts and tissue type; it was found that there is no significant difference  $P > 0.05$ . In conclusion, *Sarcocystis* spp. It is found naturally in sheep farms in the state of Jalisco. Although the



prevalence is low, its presence should be considered as a potential risk to public and veterinary health as it is a zoonosis; therefore, when performing the macroscopic identification of cysts, it is considered a first step, to be able to implement other diagnostic techniques that allow determining the species present in the area; with the aim of establishing control programs that are useful to producers.

**Keywords:** Parasite, Protozoan, macroscopic diagnosis, cysts, Mexico.

## PA-P27

### Acaricidal activity of commercial topical formulations against Cattle tick (*Rhipicephalus microplus*) from Lower Amazon

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**Objectives:** The objective of this work was to evaluate the efficiency of three topical acaricides (Amitraz, Cypermethrin, and Deltamethrin) in the control of the cattle tick (*Rhipicephalus microplus*) from the Lower Amazon region.

**Material & Methods:** To carry out the study, adult ticks (engorged females) were obtained from a commercial farm located in the municipality of Santarém, Pará, western Amazon (02°10'17" S; 56°44'42" W). The engorged ticks were manually removed from cattle without receiving acaricidal treatment for the past 60 days. The ticks were stored in plastic tubes with the lid pierced for air circulation and sent to the laboratory for the adult immersion test, which was performed on the same day of tick sampling.

#### Adult immersion test

For the adult immersion test, a total of 150 engorged female ticks were selected, washed in running water, and dried with paper towels. The test was performed according to classic protocol. The 150 ticks were divided by weight into 15 Petri dishes with 10 ticks each (1.9 ± 0.4 mg). Of the 15 Petri dishes, 4 were used for each of the 3 acaricidal drug treatment groups and 3 were used as a negative control. Three commercial products with Amitraz (125 g/L), Cypermethrin (150 g/L), and Deltamethrin (25 g/L) were used.

For the adult immersion test, the acaricidal concentration was used according to manufactures indications. Drugs were diluted and homogenized using distilled water. Final concentration used was: Amitraz: 0.125 g/mL, Cypermethrin: 0.15 g/mL, Deltamethrin: 0.025 g/mL.

Females ticks from each group were submerged in the designated solution for 5 minutes, and then removed and dried with paper towels and returned to the same plate. The control group was submerged in distilled water.

These plates were incubated in a B.O.D. (Biological Oxygen Demand) with a temperature of 28°C (±1°C) and relative

humidity ≥ 80%. For adult mortality evaluation, ticks were examined after 24 hours with counts of live and dead ticks.

The engorged females were kept in the Petri dishes under the controlled environment until the oviposition. After oviposition, the eggs were weighed and separated into syringes. The tick egg hatchability was visually estimated by trained personnel.

To determine if *Rhipicephalus microplus* is resistant to treatment, we first calculated the estimated reproduction index (ER), where ER = egg mass/female weight before oviposition X hatching percentage X 20,000. Then, acaricidal efficacy was calculated using the mean values of the plates from control and treated groups resulting in the *in vitro* acaricidal efficacy using the formula:

Acaricidal efficacy (AE) = (mean ER control group – mean ER treated group) / mean ER control group X 100.

**Results:** The topical acaricidal drugs tested presented limited adult (engorged female) mortality with 0, 2.5 and 7.5% adult mortality for Deltamethrin, Amitraz, and Cypermethrin, respectively. The negative control group had zero adult mortality.

Table 1 presents the complete evaluation of tick oviposition, egg hatchability, the estimated reproduction index, and the *in vitro* acaricidal efficacy.

Groups	Mean tick weight (mg)	Mean egg mass (mg)	ER	Mean egg hatchability (%)	AE (%)
Amitraz	176.5	13.3	7.1	53.7	90.5
Cypermethrin	195.2	84.4	42.7	88.7	10.4
Deltamethrin	202.9	79.7	39.6	80.0	26.6
Control	184.2	80.2	42.3	98.3	0.0

ER: Estimated reproduction index. AE: Acaricidal efficacy.

The egg hatchability was partially impaired by acaricidal, with Amitraz presenting lower results among the drugs tested. The Amitraz group was the most effective compared to the other two with 90.5% AE, however, considering the FAO regulations for chemical acaricidal drugs, Amitraz was not effective, since it had effectiveness lower than 95%.

The pyrethroids (Cypermethrin and Deltamethrin) presented limited acaricidal effectiveness indicating the tick has already developed drug resistance to this class of acaricidal drugs.

**Conclusion:** Among the tested acaricides, Cypermethrin, Deltamethrin, and Amitraz showed efficacy below 95% and cannot be considered effective to control cattle ticks. *R. microplus* from the Lower Amazon presented high resistance to pyrethroids. As far as we know, this is the first report to evaluate tick resistance to commercial topical acaricides in the Lower Amazon region.

**Keywords:** Ectoparasites, Amitraz, Cypermethrin, Deltamethrin, acaricidal efficacy.