Frequency of ocular pathologies in cattle (*Bos taurus*) of the department of Córdoba, Colombia

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Objetives: Veterinary ophthalmology is a relatively new specialty, being little applied in bovine medicine, so it is uncommon for bovine eyes to be examined if they do not have lesions large enough to be obvious, but it should be considered that eye problems in cattle affect the productivity and welfare of animals, since if they are not treated in time they can cause visual impairments. This study aims to report the frequency of presentation of ocular and peri-ocular diseases present in cattle from the department of Córdoba, Colombia.

Materials and methods: A study was conducted where 124 clinical cases of cattle of all races, and of both sexes, were evaluated. Descriptive statistics were performed and a distribution of macroscopically characterized ocular and peri-ocular lesions was performed.

Results: It was found that the most frequently affected ocular structure was the cornea (53.2%), followed by eyelids (17.7%). The most frequently diagnosed ocular alterations in descending order were: superficial keratitis (20.2%), corneal ulcer (16.9%), conjunctivitis (14.5%) and cell carcinoma (9.7%). The most affected sex corresponded to males (61%).

Conclusions: This is the first report of ophthalmic alterations in cattle made in the Department of Córdoba and Colombia and evidences a high presence of ophthalmic alterations, making this work an important contribution on the epidemiological state of the different ocular alterations in bovines.

Keywords: Squamous cell carcinoma, ophthalmology, keratitis.

Comparison between efficacies of intravenous hypertonic sodium chloride or bicarbonate solutions followed by intraruminal water for acute rumen lactic acidosis treatment

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The aim of this study was to compare the efficacy of intravenous (IV) administration of 7% hypertonic sodium chloride (HSC) and 6% hypertonic sodium bicarbonate (HSB) followed by intraruminal water administration for acute rumen lactic aci-

dosis treatment (ARLA) in sheep. Six crossbred adult, healthy, non-pregnant and non-lactating sheep were used. The mean body weight (BW) was 50.62 ± 5.62 kg. The ewes were submitted to ARLA induction with 15 g/kg BW sucrose diluted in water and administered into the rumen via stomach tube after 18 h of solid fasting. Each sheep was submitted to induction twice, with the second induction done at least 30 days after full recovery from the first. Eighteen hours after induction was performed rumen lavage with 20 L of water. The HSC (4 mL/kg BW) or HSB (6.7 mL/kg BW) solution was IV administered over five minutes, followed by intraruminal water, administered via stomach tube in a volume corresponding to 8% of BW. The cross-over design was used, and all sheep received both solutions, one during each induction. Physical examinations and venous blood samples were collected before (0 h), immediately after infusion (5 min) and after the treatment (48 h). The variables pH, HCO_3^- and BE were measured and strong ion difference (SID), anion gap (AG) and percent change in plasma volume (PV) were calculated. Two way repeated measures analysis of variance was used to test the effects of time and treatment, and to evaluate the interactions between them. The Tukey test was used for multiple comparisions, when the F statistic was significant. P-values <0.05 were considered statistically significant.The induction protocol was effective in causing the disease and all sheep had rumen atonia (with rumen pH < 5.0), watery diarrhea and mild-to-moderate dehydration. The ewes had metabolic acidosis at 0 h with reductions of pH (mmol/L) (HSC: 7.201 ± 0.087; HSB: 7.245 ± 0.084), HCO_3^- (mmol/L) (HSC: 14.63 ± 2.37; HSB: 17.18 ± 2.64), BE (mmol/L) (HSC: -13.37 ± 3.76; HSB: -10.15 ± 3.90), SID (mmol/L) (HSC: 37.12 ± 1.27; HSB: 37.22 ± 1.83) and AG (mmol/L) elevation (HSC: 22.47 ± 1.89; HSB: 20.07 ± 2.28). The dehydration was observed with PV (%) decreases (HSC: -3.84 ± 6.83; HSB: -8.23 ± 4.70). The solutions infusion caused exacerbations of acid base imbalances with alterations in pH (HSC: 7.098 ± 0.060; HSB: 7.596 ± 0.036), HCO_3^- (HSC: 11.38 ± 1.53; HSB: 41.92 ± 3.39), BE (HSC: -18.28 ± 2.47; HSB: 20.30 ± 3.70), SID (HSC: 30.54 ± 2.37; HSB: 53.72 ± 3.03), AG (HSC: 19.15 ± 1.93; HSB: 11.78 ± 2.02) and increase in PV (HSC: 26.58 ± 9.76; HSB: 27.43 ± 9.17). The animals presented 48 h after treatment the following values: pH (HSC: 7.259 ± 0.097; HSB: 7.375 ± 0.049), HCO_3^- (HSC: 16.90 ± 2.31; HSB: 22.77 ± 3.15), BE (HSC: -10.17 ± 3.68; HSB: -2.40 ± 3.86), SID (HSC: 32.56 ± 2.42; HSB: 35.92 ± 0.92), AG (HSC: 15.65 ± 2.28; HSB: 13.15 ± 2.56) and PV (HSC: 1.20 ± 4.77; HSB: -2.89 ± 2.74). The results of PV confirmed the water imbalance correction in both protocols treatment. There was metabolic acidosis correction after 48 h of treatment using HSB, because pH, HCO_3^-, BE and AG returned to their originals values. The animals still presented metabolic acidosis at the same period with HSC protocol, as a result values of pH, HCO_3^-, BE and SID were not returned to their baseline values. The HSC causes iatrogenic hyperchloremic acidosis, nevertheless it was effective to correct the water imbalance. The ewes were accompanied throughout the time and the acidemia was corrected after 120 h of the treatment with HSC. Despite the HSB protocol corrected the metabolic acidosis earlier than HSC protocol, the animals showed clinical recovery, that is return of appetite and forestomach motor function, at the same time with both solutions. The both protocols corrects dehydration and mild to moderate metabolic acidosis in sheep with ARLA.

Keywords: Fluid therapy, hyperosmotic solution, 7% hypertonic saline solution, 6% hypertonic bicarbonate solution.

Clinical characterization of hemoparasitism by *Babesia* spp. and *Anaplasma* spp. in Wagyu cattle

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Objective: To describe the clinical signs, hematological, metabolic, electrolytic and acid-base alterations in Wagyu cattle with hemoparasitism due to *Babesia* spp. y *Anaplasma* spp. admitted to the Large Animal at the Veterinary Teaching Hospital, School of Veterinary Medicine, Universidad Nacional de Colombia (CGA-UN).

Materials and methods: All the records of bovine cases admitted to the CGA-UN between august 2017 and April 2018 were evaluated. The inclusion criteria in the study were: 1. That the patients were of Wagyu breed 2. They had clinical signs of hemoparasitism and were confirmed by demonstration of either or both *Babesia* spp. and/or *Anaplasma* spp. in peripheral blood smear. Each patient that met the inclusion criteria had signalment, history, clinical signs, clinical laboratory tests results, rule outs, treatment and outcome collected from its medical records.

Clinical characterization was based on the clinical signs at presentation and during hospitalization. Their frequency of presentation in the patients that met the inclusion criteria was determined. Descriptive statistics that included maximum, minimum range values and standard deviation were determined.

Results: During the study period 22 wagyu cattle were admitted to the CGA-UN and 10 patients met the inclusion criteria; the prevalence of hemoparasitism in this population was 45.4%. All the affected patients were older than 12 months of age. *Babesia spp* was found in 60% of the patients and the other 40% were positive for *Anaplasma* spp. The observed parasitemia irrespectively of the agents ranged between 0,1 y 12%. The history of these patients indicated that 90% (n=9) were imported from USA within the last 6 months before admission.

The observed clinical signs in the affected animals of which males and females were in equal numbers (n=5) included: Diarrhea in all cases, ruminal and intestinal hypomotility (90%), tick presence, depression, pale mucous membranes, dehydration and increased capillary refill time in 70% of the affected animals, followed by tachycardia, pulmonary crackles in 60% of the cases and in a lower frequency low body condition, anorexia, weakness, jaundice, pigmenturia, tachypnea and oral petechiae.

Hematology tests indicated severe anemia in four patients with PCV between 10-11%, while the rest had PCV between 25 and 38%. The animals with the lower PCVs were positive for *Anaplasma* spp. Total plasma proteins were variable, they ranged from hypoproteinemia (n=2) to hyperproteinemia (n=2),

only one patient had hyperfibrinogenemia (1.0g/dL). Albumin levels were between normal ranges. Immature red cells were observed in 50% (n=5) of the cases. The white cell counts showed from leukopenia (3.400-4.200 cel/μL) in 20% of the cases to leukocytosis (12.800-25.000 cel/μL) in 50%, neutropenia was seen in 20% of the patients (884-1.112cel/μL), 30% had neutrophilia (9.396-11.750 cel/μL), 20% had lymphopenia (2.312 cel/μL) and 50% lymphocytosis (11.500cel/μL).

The clinical chemistry tests showed increased serum values of urea, creatinine, AST, GGT, glucose, lactate, and bilirrubins were increased to different values. Some of the subjects had acute renal failure. Ionic hypocalcemia and hypokalemia were present in all the animals. Arterial or venous blood gases and acid base status were evaluated indicating that 4 had a metabolic acidosis, hypocarbonetemia and increased base deficit. Three only had a mild respiratory alkalosis.

The patients were treated according to the agent and observed signs. *Anaplasma* spp. infection was treated with oxytetracycline and *Babesia* spp. with diminazene, hydration was done with saline 0.9%, metabolic acidosis was corrected with $NaHCO_3^-$ at 5% according to base deficit, fluid therapy according to dehydration, hematinics, and blood transfusion was done in cattle with PCV lower than 15%, animals with respiratory crackles were treated for pneumonia. The duration of the treatment was according to severity and duration of the clinical signs. Only one patient died possibly due to the severity of the anemia and systemic compromise at admission.

Conclusion: This is the first report of hemoparasitism in Wagyu cattle in Colombia. The anemia was more severe in Wagyu cattle infected with *Anaplasma* spp., the clinical treatment of the cases was highly successful and prevented an important mortality.

Keywords: Wagyu cattle, Anaplasma spp., Babesia spp., hemoparasitism.

Alkalizing effect of oral electrolyte solutions containing sodium acetate or bicarbonate in diarrheal newborn calves

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Objectives: The aim of this study was to compare the effects of two oral electrolyte solutions (OES), containing sodium bicarbonate or sodium acetate, as alkalizing agents, on the hydroelectrolyte and acid-base balance of newborn calves with induced osmotic diarrhea.

Material and methods: Ten healthy Holstein calves, male, with two to three days old, with effective passive immunity transfer, remained in adaptation until reaching 10 days old, ingesting milk in volume referring to 12% of body weight (BW), divided into two daily meals, with water and coast cross hay *ad libitum*. At ten days, they were submitted to osmotic diarrhea induction protocol, using milk (16.5 ml/kg) and sucrose (4 g/kg)

in 20% solution, spironolactone (2 mg/kg) and hydrochlorothiazide (2 mg/kg), every 8 hours, for two days. The calves were divided into two groups with five animals each, wich received the solution cointaining sodium acetate (SA: Glutellac®, Bayer Animal Health, Germany) or sodium bicarbonate (SB: Nutronlyt®, Nutron Alimentos, Brazil). On the day of treatment, each group received six liters of OES, divided into two feedings, at 11 am and 7 pm, using a bottle. Each OES was prepared according to the manufacturer's recommendations. Physical examinations were performed every 8 hours throughout the experimental period. Venous blood samples were collected at 48h (start of induction), 0h (start of treatment) and 72h, to measure pH, HCO_3^-, BE, Na^+, K^+, Cl^-, plasma total protein (PTP) and L lactate. Strong ion difference (SID_3), anion gap (AG), weak non-volatile acids (A_{tot}) and percentage change in plasma volume (PV) were calculated. Two way repeated measures analysis of variance was used to test the effects of time and treatment, and to evaluate the interactions between them. The probability of error of 5% was admitted.

Results: The induction protocol was effective in causing diarrhea with hydroelectrolyte and acid-base imbalances, evidenced by the reduction in skin turgor, decreased suction reflex, presence of enophthalmia, apathy and increased capillary filling time, varying in different degrees according to each calf. Before the diarrhea induction (-48h), the calves presented the measured variables within the normal range for the specie, for both SA and SB groups. At the beginning of treatment (0h), some variables underwent changes, such as pH (SA: 7.12 ± 0.11; SB: 7.21 ± 0.13), HCO_3^- (mmol/L) (SA: 13.98 ± 6.42; SB: 17.3 ± 5.48), BE (mmol/L) (SA: -15.28 ± 8.06; SB: -10.46 ± 7.64), Na^+ (mEq/L) (SA: 121.4 ± 7.31; SB: 124.62 ± 3.31), PTP (mg/dL) (SA: 6.8 ± 0.89; SB: 6.68 ± 0.86), L lactate (mmol/L) (SA: 1.11 ± 1.04; SB: 0.79 ± 0.29), SID_3 (mmol/L) (SA: 29.76 ± 6.91; SB: 33.17 ± 3.80), AG (mmol/L) (SA: 15.79 ± 1.51; SB: 15.88 ± 3.56) and A_{tot} (mmol/L) (SA: 27.88 ± 3.66; SB: 27.38 ± 3.55). PV (%) was -8.98 ± 10.37 and -15.22 ± 4.81 for the SA and SB groups, respectively. This results indicate dehydration, hyponatremia and metabolic acidosis. K^+ and Cl^- have not changed. At the end of the experiment, all values returned to baseline, with no statistical difference between the initial (-48h) and final (72h) values of any variable or OES. The animals presented 72h after treatment the following values: pH: (SA: 7,34±0,13; SB:7,32±0,14), HCO_3^- (mmol/L) (SA: 24,74±8,54; SB: 22,9±8,90), BE (mmol/L) (SA: -0,98±10,75; SB: -3,12±11,33), Na^+ (mEq/L) (SA: 130,34±6,20; SB:129,26±4,29), PTP (mg/dL) (SA: 6,12±0,30; SB: 5,76±0,47), L lactate (mmol/L) (SA: 0,87±0,53; SB: 0,47±0,11), SID_3 (mmol/L) (SA: 37,47±5,38; SB: 35,04±6,08), AG (mmol/L) (SA: 12,73±3,41; SB: 12,14±2,88), A_{tot} (mmol/L) (SA: 25,09±1,24; SB: 23,61±1,95) and PV (%) (SA: 0±0; SB: -2,07±5,12).

Conclusions: Both OES, with different alkalizing agentes (acetate and bicarbonate), used in the treatment of induced osmotic diarrhea in newborn calves, were equally effective in correcting water, electrolyte and acid base imbalances, since all parameters returned to normal at the end of the experiment.

Keywords: Osmotic diarrhea, fluid therapy, hydroelectrolyte imbalance, alkalizing agents.

Hydroelectrolytic and acid-base imbalance in calves with induced osmotic diarrhea fed with milk

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Objective: The aim of the present study was to evaluate the changes in the hydroelectrolytic and acid-base balance in newborn calves with induced osmotic diarrhea fed with milk.

Materials and Methods: Twenty-six Holstein calves, 10 days old, with 43.48 ± 3.90kg body weight (BW), fed with milk in a daily volume corresponding to 12% BW, were submitted to induction of osmotic diarrhea using protocol based on the intake of milk (16.5 mL/kg) and sucrose (4 g/kg, diluted in water, 20% solution) and the administration of diuretics, spironolactone (2 mg/kg) and hydrochlorothiazide (2 mg/kg), every 8 hours for a period of 48 hours. Venous blood samples were collected in three moments: -48h (start of the induction protocol), -24h (middle of induction protocol) and 0h (end of the induction protocol). The blood gas analysis consisted of blood pH, pCO_2, HCO_3^-, BE, Na^+, K^+ and Cl^- measurements. Hematocrit (HTC) was determined by the microhematocrit method and plasma total protein (PTP) concentrations by the refractometry method. Total concentration of weak non-volatile acids (A_{tot}), percentage change in plasma volume (PV), strong ions difference (SID_3) and anion gap (AG) were calculated. Analysis of variance of repeated measures was used to test the effect of time and a 5% probability of error was admitted.

Results: The protocol used was efficient to cause diarrhea in the studied calves, evidenced by the reduction of the skin turgor, decrease in the suction reflex, presence of enophthalmia and apathy and increased capillary filling time, varying in different degrees according to each calf. The values of HTC, PTP, A_{tot} and AG, at the time -48 h, were within the normal range (HTC: 34.15 ± 3.17%, PTP: 5.82 ± 0.51 g/dL, A_{tot}: 23,87 ± 2.09 mmol/L, AG: 11.90 ± 1.83 mmol/L) and increased in diarrheal calves from the time -24h (HTC: 39.11 ± 4.53%, PTP: 6.81 ± 0.67 g/dL, A_{tot}: 27.22 ± 2.67 mmol/L, AG: 15.05 ± 2.71 mmol/L), remaining altered at the end of the induction protocol (HTC: 40.50 ± 4.77%, PTP: 6.64 ± 0.65 g/dL, A_{tot}: 27.91 ± 2.76 mmol/L, AG: 15.91 ± 3.00 mmol/L). The reduction in %PV from the moment -24h (-12.05 ± 2.67) indicates the high loss of circulating volume. These changes together are indicative of the dehydration that the calves experienced. Severe acidemia was observed in the end of the induction protocol (pH = 7.175 ± 0.120). As a compensatory response, hyperventilation and consequent decrease in pCO_2 (39.48 ± 3.64 mmHg) were established. The HCO_3^- and BE values were, respectively, 14.88 ± 4.72 mmol/L and -13.56 ± 6.60 mmol/L at 0h, which characterize a moderate degree of metabolic acidosis. Na^+ values decreased as the protocol was instituted, falling below the reference value from the moment -24h (129.7 ± 4.42 mmol/L). Cl^- underwent a significant reduction at 0h (97.11 ± 4.02 mmol/L). The K^+ concentrations did not change significantly during the induction protocol. There was a reduction in SID_3 (mmol/L) at -24h (35.35 ± 3.40) and at 0h (30.80 ± 3.90). Hyponatremia and relative hyperchloremia led to a

decrease in SID_3, characterizing a condition of strong ions acidosis or hyperchloremic acidosis. Metabolic acidosis in calves with induced osmotic diarrhea and fed with milk was more pronounced than that observed in other studies in calves fed with milk substitute.

Conclusions: The osmotic and milk-fed diarrhea induction protocol was effective, resulting in dehydration, hyponatremia and hyperchloremic acidosis in newborn calves 48 hours after its onset.

Keywords: Neonatal diarrhea, hyperchloremic acidosis, hyponatremia, dehydration.

IM-P06

Effects of postpartum treatment with oral calcium formate on serum calcium, serum metabolites, and the occurrence of diseases in early lactation of dairy cows

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Objectives: This study aims to evaluate the effects of oral administration of calcium (Ca) formate in the postpartum of high-producing dairy cows on calcemia, on other blood biochemical markers and on the occurrence of diseases at the beginning of lactation.

Materials and methods: 120 healthy Holstein cows, distributed according to parity (first, second, third and fourth to sixth lactation), were treated or not with oral Ca formate (two doses: after calving and 24 hours later; equivalent to 50 g of Ca per dose; in a commercial liquid presentation), comprising 8 groups (n = 15). The cows belonged to the same farm, were handled in intensive system (compost barn), and received a low DCAD diet (3.33 mEq/100g DM) in the last three weeks before calving. BCS between 3.0 and 3.5 at calving and urinary pH between 6.2 and 6.8 in the week before calving were the inclusion criteria established for the study. Cows that had twin pregnancies or needed birth assistance were not included in the study. The following variables were measured in the blood serum of samples collected after calving (0h) and 24, 48, 72, and 96h after: total Ca, phosphorus, magnesium, non-esterified fatty acids, beta-hydroxybutyrate (BHB), glucose, total protein, albumin, urea, aspartate aminotransferase and gamma-glutamyltransferase. Blood BHB was also measured at 7, 14, and 21 DIM. For the assessment of disease occurrence, cows were distributed in treated (n = 60) and untreated (n = 60) and in hypocalcemic (n = 71) and normocalcemic (n = 49). Two-way repeated measures ANOVA and chi-square test were used for comparisons. This research proposal was previously approved by the institutional Ethics Committee on the Use of Animals (CEUA-UEL), under protocol number 13822.2017.94.

Results: parity did not influence the studied blood constituents, except for the highest calcemia in primiparous cows. Serum Ca was lower up to 24h and increased after 48h and subclinical hypocalcemia (SCH) ([Ca] <8.5 mg/dL) was more frequent in cows with a higher number of lactations. Treatment with Ca formate had no effect on the variation of serum Ca and the other studied variables in the first days of lactation. Treated and untreated cows did not differ in terms of milk production at 21 DIM (28.8±8.3 vs. 30.9±7.3 L; P=0.140), the presentation of diseases at the beginning of lactation (55.5% vs. 56.7%; P=1.000), the rate of discard (22.7% vs. 16.7%; P=0.263) or death (6.7% vs. 6.7%; P=1.000) up to 60 DIM, and the frequencies of SCH in the first days of lactation (60% vs. 58%; P=1.000), and of subclinical ketosis up to 21 DIM (43.3% vs. 53.3%; P=0.361). Hypocalcemic cows became more ill (64.8% vs. 42.9%; P=0.028) and had a higher frequency of retained fetal menbranes (43% vs. 20.7%; P<0.001).

Conclusions: Oral administration of Ca formate after calving and 24 hours later is not justified as a preventive measure to be adopted indiscriminately in dairy herds. Studies involving larger numbers of observation may clarify whether the selective treatment of cows with a higher risk of hypocalcemia is advantageous.

Keywords: Metabolic disorders, subclinical hypocalcemia, calcium homeostasis, transition period.

IM-P07

Prepartum anionic diet induces hyperchloremic acidosis in high-producing dairy cows without preventing subclinical hypocalcemia

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Objectives: The objectives of the study were to evaluate the effects of the pre-partum anionic diet on the electrolyte balance and calcemia of high yielding dairy cows in the first days of lactation, and to verify the impact on the frequency of subclinical hypocalcemia (SCH).

Materials and methods: 60 healthy Holstein cows, producing 30 kg of milk/day, were distributed in groups (n=15) according to parity: first, second, third, and fourth to sixth lactations. The cows belonged to the same farm, and were handled in intensive system (compost barn) receiving total mixed ration. BCS between 3.0 and 3.5 at calving was the inclusion criteria established for the study. Cows that had twin pregnancies or needed birth assistance were not included in the study. In the last three weeks before calving they received a diet with negative DCAD (-6 mEq/100g DM) and high chloride content. After calving, they received a diet with positive DCAD

(18 mEq/100g DM). Urine pH was measured in the last week before calving. Serum concentrations of sodium (Na^+), chloride (Cl^-), potassium (K^+), and total calcium (Ca), and strong ion difference (SID_3) were determined in samples taken soon after calving (0h), 24, 48, 72 and 96h after. The frequencies of SCH were determined considering the critical value of 8.5 mg/dL. Two-way repeated measures ANOVA and chi-square test were used for comparisons. This research proposal was previously approved by the institutional Ethics Committee on the Use of Animals (CEUA-UEL), under protocol number 13822.2017.94.

Results: the cows eliminated acidic urine before calving (6.65±0.30) without differences between groups (P=0.062). Na^+, K^+, Cl^-, and SID_3 values did not differ between groups. Na^+ and K^+ did not vary between days; Cl^- was elevated at calving (111.21±0.51 mmol/L) and decreased (P<0.001) until 72h (105.96±0.51 mmol/L); and SID_3 was reduced at calving (36.51±0.69 mmol/L) and increased (P=0.005) up to 48h (39.32±0.69 mmol/L). The Ca levels were reduced until 24h (8.26±0.11 mg/dL) and increased (P<0.001) up to 72h (8.62±0.11 mg/dL). Cows of third and fourth to sixth lactations presented lower values up to 24h (P=0.007). SCH was observed in almost half of the cows until 48h (48.3% at 0h, 55% at 24h, and 43.3% at 48h). The maintenance of SCH for two or more consecutive days was observed in 58.3% of cows with no distinction between groups (P=0.257): 40% in primiparous, and 53.3%, 73.3%, and 66.7% in second, third, and fourth to sixth lactations cows, respectively. The maintenance of SCH for three or more consecutive days occurred in 53.3% of third and fourth to sixth lactations cows, and was less frequent (P=0.004) in primiparous (6.7%) and in second lactation cows (13.3%).

Conclusions: Ingestion of a high chloride prepartum anionic diet led to hyperchloremic acidosis and this imbalance was reversed on the second postpartum day. The induced effects on electrolyte and acid-base balances were not able to prevent the occurrence of SCH in the first days of lactation.

Keywords: Transition period, serum electrolytes, ammonium chloride, calcium metabolism, serum calcium.

IM-P08

Effect of blood contamination on total nucleated cell count and protein concentration in cerebrospinal fluid from recumbent cows

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Many etiologies can be the cause of the downer cow syndrome. Among them, spinal cord lesions are usually associated with a poor prognosis. Analysis of cerebrospinal fluid (CSF) is a useful procedure to establish a diagnosis and prognosis. Red blood cells (RBC) are not a normal component of CSF. Total nucleated cells count (TNCC) and protein concentration (TPC) could be falsely elevated in the presence of blood contamination.

The aim of this retrospective study was to describe the variation of TPC, TNCC and RBCC using CSF analysis results from recumbent dairy cows admitted to the Centre Hospitalier Universitaire Vétérinaire.

Among the 235 samples included, RBCC varied between 0-869220 cell/µL (mean=4741.9, median=6.6), TPC varied between 0.04-6.51 g/L (mean=0.43, median=0.27), and TNCC varied between 0-7500 cell/µL (mean=35.5, median=1.1).

One hundred and seven samples had <30 RBC/µL. Among them, TPC and TNCC varied between 0.13-1.06 g/L (mean=0.29, median=0.27) and between 0-31.4 cell/µL (mean=1.8, median=0.6), respectively.

Eighty-four samples were less likely to be associated with a spinal cord lesion (TPC <0.25 g/L, TNCC ≤4.5 cell/µL). Among them, RBCC varied between 0-1290 RBC/µL (mean=61.5, median=4.7).

Twenty samples had absence of nucleated cells (TNCC=0 cell/µL). Among them, RBCC varied between 0-840 RBC/µL (mean=65.8, median=3.9).

A RBCC of <840 RBC/µL seems to less likely interfere with CSF results interpretation. The threshold of 30 RBC/µL (used in humans) appears to low in bovine. To study the TPC and TNCC levels of CSF samples before and after known dilutions of whole blood are added is suitable.

Keywords: Cattle, downer cow, cerebrospinal fluid analysis, blood contamination.

IM-P09

First quantification of propionic acid in serum of dairy cows from different farms

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Objectives: Propionic acid is produced by microbial fermentation of carbohydrates in the rumen of cattle (up to 54 mol/day). The liver metabolizes up to 75 % of absorbed propionic acid from the rumen and up to 25 % is directly metabolized by mammary gland tissue. Propionic acid is converted to glucose by two key enzymes (Propionyl-CoA Synthetase, EC 6.2.1.17; Propionyl-CoA Carboxylase, EC 6.4.1.3). The inhibition of gluconeogenic enzymes by phenolic substances in vitro was found in ovine hepatocytes. Therefore, it is possible that propionic acid accumulates in bovine blood by continuous high production in the rumen. Both very low and high concentrations of propionic acid in peripheral venous blood can be expected. No specification concerning levels of propionic acid in blood except experimental data are available from previous studies. The aim of this study was to analyze the variability of propionic acid in serum of dairy cows from different farms by gaschromatography.

Materials and methods: A total of 360 serum samples

from dairy cows were examined for the presence of propionic acid. Of these samples, 180 were from chronically sick and 180 from healthy animals of various control- and case-farms. Case-farms are dairy farms with a chronic, unspecific disease process.

The method is based on the derivatization of propionic acid by reaction with 2-chloroethyl chloroformate (2). The internal standard n-valeric acid was added to the serum and was deproteinized with highly concentrated hydrochloric acid and subsequently centrifuged. For the following treatment, samples were alkalized with sodium hydrate and vacuum-dried. Afterwards the reaction medium consisting of pyridine, acetonitrile and 2-chloroethanol was added to the dried sample. The 2-chloroethyl ester of propionic acid is formed by addition of 2-chloroethyl chloroformate. In the ultimate step, the derivative was extracted by chloroform and analyzed by gaschromatography.

N-valeric acid was developed as internal standard (determined limit of detection for propionic acid: 45.26 µmol/l; CV in series for n-valeric acid: 5 %; CV in series for propionic acid: 4.4 %). In addition, all samples with propionic acid contents under 45.26 µmol/l were analyzed with a modified method that aimed at lowering the detection limit. In these samples, the concentration of propionic acid ranged from 0.0001 to 0.004 µmol/l. The statistical analysis was performed with SAS Enterprise Guide (Wilcoxon's two-sample Test). To determine the reference limits for propionic acid the internationally acknowledged procedure of the IFCC (3) was used. The reference interval should contain the central 0.95 fraction (or 95 %) of the reference distribution.

Results: There were no significant differences in propionic acid concentrations between diseased and healthy cattle within one case and control farm (P>0.05). There were significant differences in propionic acid concentrations between diseased cattle within different case and control farms as well as between healthy cattle within different case and control farms (P<0.05).

The 2.5th and 97.5th percentile of the whole sample collective incorporates propionic acid concentrations from 0.01 to 1063 µmol/l.

Conclusions: The established method is cost-efficient (no ultra-filtration) and allows easy preparation and rapid processing. Under the given conditions, a first potential normal range for propionic acid in serum of cattle was elaborated: 0.01-1063 µmol/l. The high variability of propionic acid contents in the investigated collective can be explained by the factors that affect propionic acid levels in blood: the composition of the diet, the dry matter intake and the time of sampling after feed intake. Further studies are necessary to investigate the importance of those factors in order to define proper sampling schemes and to further examine the role of propionic acid as potential biomarker for animal health.

Keywords: Propionic acid, serum, dairy cows.

Bioactive compounds in organic and aqueous extract in plant species of Mexico

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The aim of this study was to identify the total phenolic compounds of organic (methanolic and butanolic) and aqueous extracts and evaluate their biological activity. The presence of phenolic compounds was determined by the technique of gelatin-sodium chloride, as well as the recognition of flavonoids with the Shimoda technique. Total phenols were quantified by ultraviolet light spectrophotometry and Folin-Cicalteau reagent, using the tannic acid standard, which results were expressed in mg of tannic acid/g. Thin layer chromatography was used to identify the flavonoid presents in each extract. The antioxidant activity was evaluated in the extracts with higher polyphenol content, using the commercial Trolox kit expressed as µMol / 100g. The results indicated that tejocote methanolic extract and peanut dichloromethane extract had a higher phenolic content of the condensed tannin group (759.12 mg / g; 103.61mg / g) and red corn with the lowest condensed tannin content (68.0 mg Tannic Ac / g) in the butanolic extract. It is concluded that the solvent used in the extraction of polyphenolic compounds is very important as well as the type of sample and its genotype, since methanol was better for the extraction of total phenols in tejocote; water for red corn and blue corn and dichloromethane for peanuts. The highest content of flavonoids was obtained in the tejocote leaves, comparing to blue and red corn and peanut. Chromatography allowed the identification of quercetin (Rf = 0.91) and hesperetin (Rf = 0.96) as the flavonoids present in extracts of tejocote leaves, therefor these extracts might be used for the treatment of bacterial diseases such as Salmonellosis, Colibacillosis, Listeriosis and Mastitis produced by *Staphylococcus aureus* or *Pseudomonas aeruginosa*. The antioxidant capacity of the tejocote leaves extract was 0.46 mmol Trolox / g, higher than vitamin E (0.20). The peanut had a high inhibitory activity of 0.65 mmol Trolox / g higher than vitamin C and vitamin E.

Keywords: Bioactive compounds, plant species, antioxidant activity.

Determination of Potassium Levels in Cows with metabolic diseases and in the Periparturient Period

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Objective: Detection and prevention of hypokalemia which causes economic losses and metabolic diseases in dairy cows have great importance. For this reason, the first objective of this study is to determine the level of blood potassium (K) in dairy cows in the periparturient period, and the second objective is to a determination of the relationship between metabolic diseases and blood potassium level.

Material and Methods: Healthy 40 cows (group I; 20 and group II;20) which are pregnant and multiparous and 20 cows with metabolic diseases (group III) were used 60 cattle totally. First blood sample was taken 2 weeks before calving, and the second blood sample was taken within two weeks after calving. Blood samples of 20 cows with metabolic diseases were taken from V.Jugularis once, as 10 ml at admission. All blood samples were centrifuged at 5000 rpm for 10 minutes. Serum samples were analyzed for Na, K, Cl, Creatinine, SGOT, LDH, Mg, P and CPK parameters.

Results: Clinical symptoms related to hypokalemia and metabolic diseases in healthy groups (I and II) were not observed in during the study. There was also no decrease in milk production and appetite. Serum potassium levels of healthy groups were mild-moderate decreased in after birth compared to before birth values. The mean potassium level of group I and II healthy cows after calving were 2,35 ± 0,17 mmol/l and 2,71 ± 0,12 mmol/l, respectively.

Hypokalemia had observed in cows with displacement of abomasum and ketosis (group III). The cows had also anorexia, weakness and decreased milk production. Mean serum potassium levels had 2,53 ± 0,06 mmol/l in cows with metabolic diseases. The potassium levels in cows with metabolic diseases (group III) had lowest compared to healthy groups.

In conclusion: development of negative K balance was detected in lactating cattle after calving. Additionally, cows with displaced abomasum and ketosis were also hypokalemic.

Keywords: Cow, hypokalemia, potassium, ketosis, displacement of abomasum.

IM-P12

An outbreak of *Vicia villosa* intoxication in a French Holstein dairy herd

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Objectives: The main objective of this report is to raise awareness among veterinarians about hairy vetch (*Vicia villosa*) poisoning in cattle through the description of an outbreak in a French dairy herd.

Material and methods: In mid-November 2019 a Holstein dairy farm located in the north-eastern part of France in the *Ardennes* reported several cases of severe hyerthermia and pruriginous dermatitis associated with high mortality rate. The cows had been kept indoors in their usual tie-stall barn since late-October 2019. A wide range of treatments, including antibiotics, anti-histamines, avermectins, steroidal- and non-steroidal anti-inflammatory drugs were administered with no avail. In late-November 2019, a 3 year-old Holstein cow – which had been sick for 12 days – was addressed to *Maisons-Alfort* Large Animal Hospital for examination and necropsy.

Physical examination revealed a wasted animal, with a mild hyperthermia (39.5°C) and superficial lymph nodes enlargement. Close examination of the skin showed multifocal alopecic scaly plaques becoming sometimes confluent, often associated with thickening of the skin on the head, neck, trunk, and escutcheon. Numerous papules and crusts were noted on the face, the limbs, the interdigital spaces and the udder. Pruritus was noted during the clinical examination. Lesions were restricted to the skin and independent of the pigmentation.

The association of pruriginous dermatitis with hyperthermia and lymph nodes enlargement in a herd of dairy cattle located indoor led to the following differential: malignant catarrhal fever (MCF), stachybotryotoxicosis, and sterile eosinophilic folliculitis and furunculosis. The implication of sarcoptic mange, dermatophytosis, dermatophilosis or staphylococcal folliculitis and furunculosis could not be excluded but could not explain the severity of this condition. In order to explore the eventuality of these hypotheses, skin scrapings, trichoscopic and skin cytologic examinations were performed and were unremarkable. MCF hypothesis was excluded based on a negative polymerase chain reaction (PCR) assay on whole blood. Skin biopsies at different locations were performed and processed for routine histopathological examination.

Results: At necropsy, besides the aforementioned skin lesions, decisive gross pathological findings concerned the liver, the heart and the lymph nodes. The liver was bulging, purplish-blue, mottled with clearly demarcated beige spots or large patches; on cut surfaces these beige patches deeply infiltrated the parenchyma . In the heart, the epicardial surface of the left atrium and of three quarters of the left ventricle was uniformly light brown. On its cut surface, the left ventricular wall was diffusively beige on a depth of 1 cm. The rest of the heart was studded with firm, grayish multifocal nodules visible on epicardial and endocardial surfaces. They were slightly protruding in the heart lumen.The lymph nodes were all markedly enlarged; on their cut surfaces there were raised multifocal nodules of 5 to 10 mm in diameter. Organ samples were processed for routine histopathologic examination.

Histopathological findings were similar for the skin, the heart and the liver and consisted in multifocal granulomatous eosinophilic inflammation with numerous multinucleated giant cells that varied in intensity from organ to organ. Comments by the reviewing pathologists were that these changes were highly suggestive of hairy vetch (*Vicia villosa*) poisoning.

Conclusion: Investigation in the farm pointed out that all the lactating cows had been grazing on an artificial pasture for two months prior to their return indoors. Unlike previous years, the farmer had mistakenly used hairy vetch seeds instead of common vetch (*Vicia sativa*). In the end, morbidity was evaluated at 17% (9/54) and lethality rate at 78% (7/9). These figures are consistent with previous reports of vetch-associated outbreaks and stress the lethality of this disease. To our knowledge, this is the first report of hairy vetch intoxication in cattle in Europe.

Vetch-associated diseases have been linked to the consumption of plants of only some species of *Vicia*: *villosa*, *dasycarpa*, and *benghalensis*, but not *sativa* which had been used on this farm for eight years before the seed supplier mistakenly changed the usual order. The farmer should have been alerted of this toxic risk but since hairy-vetch poisoning seems occasional and limited to some breeds of cattle and has never been described in Europe, it is not surprising that this information was omitted by the supplier.

Keywords: 'hairy vetch' 'systemic granulomatous disease' 'intoxication'

IM-P13

Is antimicrobial treatment for calf diarrhea a risk factor for pneumonia?

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Calf pneumonia is a major cause of morbidity and mortality in preweaning dairy calves. Risk factors include sudden changes in temperature and humidity, transportation and stress. However, the role of antimicrobial for preceding illnesses such as diarrhea as a risk factor for pneumonia remains unknown. The objective of this study was to evaluate the association between antimicrobial treatment for diarrhea with the risk of being treated for pneumonia during the first 30 days of calf life.

This study used a retrospective methodology. Calf health records from 10 dairy farms were reviewed and the following information was recorded: antimicrobial treatment for diarrhea, and antimicrobial treatment for pneumonia.

Treatment records of 4300 calves were available. The incidence of diarrhea was 76% (3271/4300). The incidence of pneumonia was 46% (1997/4300). During the study period, 59% (1923/3271) of the diarrheic calves were treated with antimicrobials. Univariable logistic regression model revealed that calves treated for diarrhea had a higher odds (Odds Ratio (OR): 1.99; P < 0.001; 95% Confidence Interval (CI): 1.73 to 2.31) of being treated for pneumonia. Additionally, of the calves that were treated for diarrhea, if they received an antimicrobial treatment as part of their diarrhea therapy, they had a 2.22 higher odds (P < 0.001; 95% CI: 1.92 to 2.57) of being treated for pneumonia.

Calves that receive antimicrobial treatment for diarrhea had higher odds of being subsequently treated for pneumonia than diarrheic calves without antimicrobial treatment. Antimicrobial therapy for diarrhea may have a detrimental effect on the respiratory microbiota potentially predisposing to infection, however this hypothesis warrants further investigation.

Keywords: pneumonia, diarrhea, antibiotics, antimicrobial stewardship.

IM-P14

Bacteria involved in sepsis in calves and their antimicrobial resistance

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Objective: Sepsis is a life-threatening condition in calves, often necessitating the use of broad-spectrum and bactericidal antimicrobials. As in human medicine, critically important antimicrobials such as cephalosporins and fluoroquinolones are often preferred for this indication. However, legislation in food-producing species warrants their prudent use. In cattle, insufficient information is available concerning the bacteria involved and their resistance profiles. Therefore, the objective of this study was to determine the occurrence of sepsis in critically ill calves, the bacteria involved and their antimicrobial susceptibility.

Material & methods: A retrospective cohort study was conducted on 230 calves in a critically ill state. Routine hemocultures on enriched aerobic medium (BACTEC™) were done in all 230 calves. The hemoculture positive critically ill animals were considered in sepsis and the isolates were analyzed, focusing on identification of etiologic agents, antimicrobial susceptibility testing, presence of multidrug resistance (MDR, ≥ 3 different classes), and determination of the appropriateness of the used antimicrobial treatment. Inappropriate antimicrobial use was defined as an antimicrobial therapy to which the pathogen was resistant, taking the intrinsic resistance against pathogens into account. (In)appropriateness was evaluated per case and for each antimicrobial.

Results: Sepsis (positive hemoculture) was evidenced in 34% (79/230) of the critically ill animals. In 67 of those animals one or more bacteria were isolated and identified to species level. In total 62.5% (45/72) Gram-negative and 37.5% (27/72) Gram-positive bacteria were found. The most frequently isolated species was *Escherichia coli* in 30.6% of the cases (22/72), followed by *Staphylococcus* spp. (15.2% (11/72)) and *Salmonella* spp. (9.7% (7/72)). Of the isolated bacteria, 61.7% of the bacteria displayed MDR, and was especially high in *E. coli* (90.9%). Treatment was appropriate in 78.7% (37/47) of the suitable cases. Inappropriate treatment of the animals with sepsis did not significantly increase their odds at mortality *(P* =0.76). When appropriate treatment was initialized, 64.9% (24/37) of the calves died, versus 70.0% (7/10) in case of inappropriate treatment. Inappropriate treatment against isolated bacteria was frequent in potentiated sulfonamides (50.8%) and aminopenicillins (65.0%), whereas it was more limited for ceftiofur (28.5%) and enrofloxacin (19.6%).

Conclusion: Hemoculture was positive in 34% of the critical ill calves, suggesting sepsis. Enterobacteriaceae were the most frequently isolated causative agents, but also Gram-positive bacteria were found. Antimicrobial resistance against aminopenicillins and potentiated sulfonamides was more prevalent in contrast to the critically important quinolones and cephalosporins. High MDR in various bacteria is worrisome and close monitoring of appropriateness of antimicrobials in the future is warranted.

Keywords: Cattle, Hemoculture, Multidrug resistance, Appropriate antimicrobial treatment.

Abomasitis associated with halofuginone intoxication in pre-weaned calves

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Objectives: Halofuginone is widely used to prevent calf diarrhea due to infections with Cryptosporidium parvum. Reported clinical signs of intoxication are diarrhea, blood in feces, anorexia, dehydration, apathia and exhaustion. Here, we report a case of long-time overdosing of halofuginone in pre-weaned calves, mainly presenting as abomasitis.

Material and methods: Seven Belgian blue beef calves (4 days – 28 days of age) from the same farm were presented to a referral clinic with complaints of anorexia and weight loss. Recently, three calves from the same farm had died after showing comparable clinical signs. Cases were examined clinically and by ultrasound. In lethal cases, gross pathology and histology were performed.

Results: All animals showed signs of dehydration, but only two animals had diarrhoea. Based on the ultrasound, four animals were diagnosed with pneumonia. Six animals had an enlarged abomasum with an oedematous wall. After administration of intravenous fluid therapy, antibiotics and proton-inhibitors four animals reached clinical cure after 7 – 19 days. On the necropsy of the other three animals an abomasitis was detected with different severities going from oedematous to haemorrhagic and ulcerative. Further histological examination of the abomasum identified lesions ranging from oedema to necrosis in the mucosa. No lesions were observed in the mouth, pharynx and oesophagus in any of the animals. Further questioning of the farmer revealed that his original dose was to high (194 µg/kg, 100 µg/kg according to leaflet recommendations) and that in response of diarrhea in the calves he further increased the dose to 389 µg/kg. After correction of halofuginone dosing, no new cases occurred.

Conclusions: Halofuginone is known to be a drug with a narrow therapeutic-margin, but abomasitis has not previously been mentioned in case of intoxication. Halofuginone intoxication should be included in the differential diagnosis of milk refusal (anorexia) in 1-3 week old calves.

Keywords: Intoxication, calves, halofuginone, abomasitis.

Comparison of the content of selected heavy metals in liver tissue of wild boar, red fox and red deer living in north-eastern Poland

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Objectives: The aim of this study was to determine the content of selenium (Se), zinc (Zn), copper (Cu) and cadmium (Cd) in the liver of predominantly plant-eating omnivore wild boar (Sus scrofa), predominantly meat-eating omnivore red fox (Vulpes vulpes) and herbivore red deer (Cervus elaphus), from North-Eastern Poland, in order to verify distribution of this elements in the trophic pyramid. Furthermore, the study was used to assess the risk of eating venison.

Material and methods: The research was carried out on three species of wild mammals- wild boar (Sus scrofa), red fox (Vulpes vulpes) and red deer (Cervus elaphus) from the region of Warmia and Mazury. The research was conducted during the hunting season from October to December 2015. Tissue samples were obtained from 10 red deer, 10 wild boars, and 10 red foxes. The animals were shot by hunters under the hunting law and did not show any external signs of disease. Liver samples (200g) were collected from the animals immediately after the shot and the concentration of Cu, Zn, Se, Cd was measured in the homogenates by flame atomic absorption spectrometry using AAS Solaar M6. The obtained results were subjected to statistical tests using the Mann-Whitney-U test with Python 3.7 software.

Results: The average concentration of Se was 3.9 (0.32 ± 0.09 mg/kg, $P<0.001$), and 1.8-fold higher in wild boar (0.14 ± 0.05 mg/kg, $P<0.001$), and red fox in comparison to red deer (0.08 ± 0.01 mg/kg) and 2.1-fold higher in wild boar comparing to red fox ($P<0.001$). There was no significant difference in the average concentration of Zn between red fox (28.86 ± 10.35 mg/kg), wild boar (30.97 ± 5.57 mg/kg), and red deer (28.60 ± 1.85 mg/kg). The average concentration of Cu was 9.3 and 5.4-fold higher in red deer (27.64 ± 9.51 mg/kg) in comparison to wild boar (2.96 ± 0.61 mg/kg, $P<0.001$) and red fox (5.06 ± 3.30 mg/kg, $P<0.001$). The average concentration of Cd was 1.9-fold higher in wild boar (0.15 ± 0.05 mg/kg) in comparison to red fox (0.08 ± 0.06 mg/kg, $P<0.029$). The average Cd level in red deer was 0.15 ± 0.01 mg/kg.

Conclusions: In conclusion we can say that the liver concentrations of selenium (Se), zinc (Zn), copper (Cu), cadmium (Cd) in selected species of wild animals from the hunting areas of Warmia and Mazury do not exceed from standard values. These results do not diverge from the literature data. A low concentration of selenium may be related to the low level of this element in the studied area. The cadmium and zinc level correspond with the low level of this heavy metal in the other parts of Poland.

Keywords: Wild animals, liver, heavy metals.

IM-P19

Comparison of the efficacy or enrofloxacine hcl-2h2o and oxytetracycline in cases of metritis in pospartum cows

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Objectives: To evaluate and compare the efficacy of the treatment of clinical metritis, through the use of enrofloxacin hydrochloride dihydrate applied intrauterine route plus prostaglandin F2 α using parenteral route in the Treated Group (A); Oxytetracycline applied intrauterine route plus prostaglandin F2α in a parenteral route in the Control Group (B).

Material and methods: The study was carried out in a commercial milk production facility, located in the State of Hidalgo, México.

The target population was 100 animals with clinical metritis. Two groups were formed, the Treated Group (A) received 3 grams of enrofloxacin HCl-$2H_2O$, diluted in 50ml of physiological saline solution, via IU and a support therapy with 2ml of prostaglandin $F_2\alpha$ using a parenteral route (only in the first treatment).

The Control Group (B) received 2.5 grams of injectable oxytetracycline via IU, and 2ml of prostaglandin $F_2\alpha$ parenterally as support therapy (only in the first treatment).

Results: Regarding the number of treatments required to achieve the healing of the animals, an average of 2.1 treatments with enrofloxacin were required, with a total of 29 cured animals at the first treatment, 13 at the second and 7 at the third for the Treated Group (A).

For the Control Group (B) with oxytetracycline, an average of 2.26 treatments were required, 13 animals responding to the first treatment, 13 to the second and 11 to the third; but there were no significant differences between groups (P> 0.1).

In relation to the reproductive parameters, it can be observed that as regards to the period of delivery at first heat in the Treated Group (A) it was 44 days on average, while in Control Group (B) it was 71.36 days on average. Therefore, from the statistical point of view, it was significant between the groups (P <0.05).

As for the period between delivery to first insemination, in the Treated Group (A) it was up to 82.33 a day on average, leaving 15 animals pregnant at the first service; while in the Control Group (B), it was 105.9 per day on average, with 10 animals being pregnant at first service. From the statistical point of view, a significant difference is shown (P <0.05).

Conclusions: The efficacy of enrofloxacin in metritis problems in Holstein Friesian cows at the first treatment was 58%, while for oxytetracycline it was 26%.

Keywords: Metritis, enrofloxacine, oxytetracycline, dairy cows.