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WORLD BUIATRICS CONGRESS

SEPTEMBER 4TH TO 8TH

MADRID 2022

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ABSTRACT BOOK

31st World Buiatrics Congress

September 4th to 8th, 2022

Madrid, Spain

Organizers:

The National Association of Spanish Specialists in Bovine Medicine (ANEMBE)

The World Association for Buiatrics (WAB)

WBC 2022 Congress Technical Secretariat:

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Results: Among biochemical parameters, only AST was significantly increased in Group - RK. A total of fifty-seven metabolites were identified in serum samples: 27 amino acids and derivates, 9 organic acids, 5 alcohols, 4 carbohydrates, 3 amine and derivates, 2 fatty acids, 2 ketone bodies, 1 sulfone, 1 vitamin, 1 imidazole, 1 nucleoside, and 1 guanidine. Six of the identified metabolites showed a statistically significance, specifically: glycerol, taurine, and creatinine showed a significant reduction in Group - RK, whereas acetone, acetate and 3-hydroxybutyrate showed a significant increase. In addition, six metabolites showed a trend toward significance: methanol, proline, and glycine were reduced in Group - RK, whereas formate, citrate, glutamate were increased. The rPCA analysis failed to cluster groups, while the PLS-DA showed two cluster principally related to acetate, 3-hydroxubutyrate, acetone, and glycerol (VIP > 1.5). The ORA analysis identified five metabolic pathways possibly responsible for changes in metabolome profile: glyoxylate and dicarboxylate metabolism; pyruvate metabolism; glycolysis / gluconeogenesis; glycerolipid metabolism and taurine and hypotaurine metabolism.

Conclusions: Metabolomic analysis through ^{1}H-NMR is a useful tool to achieve knowledge about metabolic profiling related to serum BHB modifications in dairy buffaloes. The metabolic state of our animals at risk of hyperketonemia suggests an initial mobilization of body resources, subclinical inflammation and potential oxidative stress status, changes in ruminal fermentations, influence on urea cycle and thyroid hormone synthesis. This study demonstrates that the metabolomic approach identified potential relationships with the development of subclinical ketosis even if the BHB concentration did not exceed the threshold value.

Keywords: Metabolomics; Negative energy balance; Mediterranean buffaloes; H-NMR; Ketosis.

BC-04

First detection of "*Candidatus* Mycoplasma haemolamae" in alpaca (*Vicugna pacos*) in Italy

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Objective: *Candidatus* Mycoplasma haemolamae is a wall-less hemotropic prokaryote that infects camelids. To the author's knowledge, there have been no published reports of *Candidatus* M. haemolamae infection in alpacas in Italy. This study describes a clinical case of *Candidatus* M. haemolamae infection in an alpaca cria from northern Italy and the prevalence in its herd.

Materials and methods: A 2 month-old alpaca cria was referred to the Clinic for Ruminants of the Veterinary Teaching Hospital of the University of Milan for weakness and lethargy. At birth, the alpaca cria was immediately rejected by their dam and fed with artificial bovine colostrum (Locatim®, Boehringer Ingelheim, Germany) and pasteurized whole cow's milk. At admission, the cria presented pale mucosae, tachycardia, and moderate dehydration. The hemogasanalysis underlined severe hypoglycaemia (1.1 mmol/L) and anaemia (haematocrit 17%, haemoglobin 5.6 g/dl). The complete blood count (CBC) confirmed mild, regenerative anaemia. The blood smear revealed numerous small basophilic coccoid structures attached to the surface of erythrocytes compatible with *Candidatus* M. haemolamae infection. In addition, parasitology examination of the faeces revealed severe coccidian infestation. To confirm the presence of *Candidatus* M. haemolamae, a portion of the 16S rRNA gene was amplified using a species-specific real-time PCR on blood samples collected at day of admission and at day of discharge. Threshold cycle (Ct) number was used as the measure of bacterial load (the lower the Ct level the greater the amount of target nucleic acid is present in the sample). Subsequently, the other animals of the entire herd from which the primary case was detected were tested by real-time PCR and by blood smear examination to investigate the presence of *Candidatus* M. haemolamae (n=20).

Results: In the blood sample collected at admission, real-time PCR revealed a high level of *Candidatus* M. haemolamae DNA (Ct 11.7). The cria was stabilized by administration of a 10% glucose solution, iron dextran (5 mg/kg, subcutaneously once), B vitamins (10 mg/kg, subcutaneously once), E vitamins, and selenium (0.05 mg/kg, subcutaneously once). Furthermore, the animal was treated with long acting oxytetracycline (20 mg/kg, subcutaneously, q72h for 3 treatments) for the *Candidatus* M. haemolamae infection. By the third day of hospitalization, the animal's clinical condition had improved. Eight days after hospitalization, the haematocrit (30.1%) and haemoglobin (11.7 g/dl) were also within the reference ranges, and the alpaca was discharged. The blood smear, performed on the day of discharge, did not show the presence of *Candidatus* M. haemolamae whereas real-time PCR was still positive, showing lower DNA levels (Ct 24.7) compared to the first blood sample. Anticoccidial therapy was set at discharge with sulfadimethoxine (110 mg/kg, orally, q 24 h for 10 days).

A 65% (13/20) *Candidatus* M. haemolamae real-time PCR positivity was reported in the other animals of the herd, with Ct values ranging from 16.4 to 32. A poor agreement between PCR result and smear examination was observed. The dam of the cria showed positive molecular results. An overall 66.7% (14/21) prevalence was observed in the herd, including the alpaca cria. No animal other than the cria had clinical manifestations correlated to the infection.

Conclusion: This study reports the first identification of *Candidatus* M. haemolamae in Italy. As shown in other studies, clinical infection was observed in a young animal, and further parasitic infestations (such as coccidiosis) were associated with *Candidatus* M. hemolamae infection. Treatment with oxytetracycline during *Candidatus* M. hemolamae infection was valid only for symptom remission, but the alpaca cria continued to be PCR positive after treatment, in accordance with previous observations on treatment in positive alpaca. Despite the very high prevalence of *Candidatus* M. haemolamae, most infected alpacas did not show clinical abnormalities. The absence of maternal colostrum intake suggests that the cria was not infected by colostrum. Further investigations are needed to assess the transmission dynamics of *Candidatus* M. haemolamae in Italian alpaca herds.

Keywords: Alpaca, Candidatus Mycoplasma haemolamae, Anaemia.

BC-05

Improving the embryo developmental competence and success of in vitro produced calves during hot season in Buffalo

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Heat stress is a major problem for animal breeding in tropical and equatorial area as well as in the Mediterranean countries. This translates into several pathologies and high rates of reproductive failure through early embryonic loss in buffalo. The present work was conducted to investigate:1) Effect of cold and hot season in the oocyte quality and in vitro embryo development competence of buffalo cultured in buffalo oviduct epithelial cell (BOEC).2) Effect of heat stress (41°C) & IGF during in vitro oocyte maturation in oocyte maturation rate and embryo developmental competence.3)Transfer of in vitro produced embryo during hot season to Egyptian buffalo. Egyptian buffalo's ovaries were collected during hot (June-August) and cold (December-February) season from abattoir. Oocytes were classified to Excellent (Ex), Good (G), Fair (F), Denuded (D). EX and G oocytes were matured in in-vitro maturation medium (IVM) (TCM-199+ 10% FCS + 10 µg/ml FSH+ 10 ng/ml EGF+ 50 µg/ml gentamicin (gn) for 22 h in 38.5C and 5% CO_2. The matured oocytes (1st pb) fertilized using Frozen – thawed buffalo semen, for 18 hours incubation. Zygotes cultured in SOFM + 10% FCS, 5 µg/ml insulin and 50 µg /ml gentamicin, with or without BOEC and incubated for 8 days. 2) Ex & G oocytes IVM in TCM-199 + 10% FCS+ 10 µg/ml FSH + 10 ng/ml IGF + 50 µg/ml, for 41°C /1hr- then 38.5°C vs. 38.5°C incubation for 22 hours. Then matured oocytes were fertilized and cultured as described before. Blastocyst were Fixed for cell counting to validate the quality of blastocyst. 3) Non-surgical embryo transfer during hot season in National Research Centre farm, Fresh Two in-vitro produced embryos (IVM,TCM+IGF, cultured in BOEC) were transferred to each buffaloes came in natural oestrus (buffalo number=5).Pregnancy diagnosis after 40 days from transfer. Results, Oocytes were collected from (349) buffalo ovaries giving an average of 2.4 oocytes/ovary (range 1.8 – 3.0 oocytes/ovary).There were highly significant (P<0.01) differences in the recovery rate of total oocytes between the two seasons, cold and hot season (3.02% ± 005 and 1.76% ± 0.05 respectively). Analysis of quality of buffalo oocytes revealed that, there were highly significant (P<0.01) differences in the mean % ± SE of excellent and good quality oocytes between the two seasons cold (38.63% ± 0.5, 51.35±0.50 resp.) and hot (11.29% ± 0.64, 21.37±0.74 resp.). While, fair and denuded oocytes increased with higher significant differences (P< 0.01) in summer (58.45% ± 0.86) when compared with the cold (9.86% ± 0.23). Maturation rate (85- 84 %), cleavage rate (75-71 %), blastocyst rate (33- 2%) were significantly higher (P<0.1, P<0.5) in cold temperature when compared with hot temperature season, in range 71-70%, 63-60 % and 22-17 respectively. In vitro culture of embryo using BOEC vs. without BOEC significantly increase the blastocyst rate either in cold (33 vs. 27 %) or hot temperature (22 vs. 17 %). The cell number of the blastocyst was significantly higher (P<0.1) in the cold temperature (mean= 106-90) when compared with hot temperature (80-60) and in vitro culturing in BOEC significantly increase (P<0.1) the blastocyst cell number either in cold or hot season. Effect of heat stress 41°C during in vitro oocyte maturation on in vitro embryo developmental competence in buffalo. Higher temperature 41°C for one hour during in vitro oocyte maturation in buffalo significantly (P<0.01) decreased the mean ± SD and rate of maturation (32±3.79, 51), cleavage (16.5±2.65, 26) and blastocyst (13.5±2.65, 22), when compared with in vitro oocyte maturation in 38.5°C (77.50±7.46, 87%, 42.67±2.52, 72% and 37.00±5.03, 41% respectively). Non- Surgical transfer of in vitro produced fresh buffalo embryos to five buffalos (day 6 of natural oestrus), pregnancy and calving of two calves (2/5, 40% Emy & Medo) with 42 kg body wright.

In conclusion: Heat stress either during hot season (summer) or through experimental rise temperature during in vitro maturation of buffalo oocytes significantly decrease the in vitro oocyte competence and blastocyst rate. Supplementation of IGF in in vitro maturation medium and co-culture of in vitro fertilized oocytes using BOEC decreased effect of heat stress and improved in vitro embryo production of buffalo. Success of calving of two calves (EMY& Medo) through transfer of IVP buffalo embryos matured in TCM+IGF during hot season (summer).

Keywords: Buffalo, season, BOEC, embryo, ET.

BC-06

Herd, buffalo, and quarter specific prevalence and risk factors of subclinical mastitis in water buffaloes of Bangladesh

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