Game of Research Season Four, 4June 2021

OMIC characterization of cow, donkey and goat milk extracellular vesicles reveals their anti-inflammatory and immune-modulatory potential

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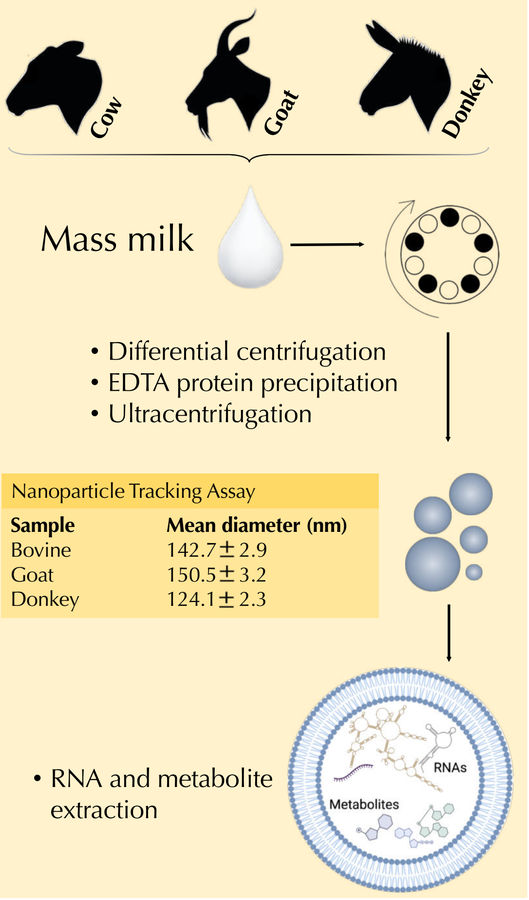
Sample collection



Nanoparticle Tracking Assay

Sample	Mean diameter (nm)
Bovine	142.7±2.9
Goat	150.5±3.2
Donkey	124.1±2.3

Morphological characterization

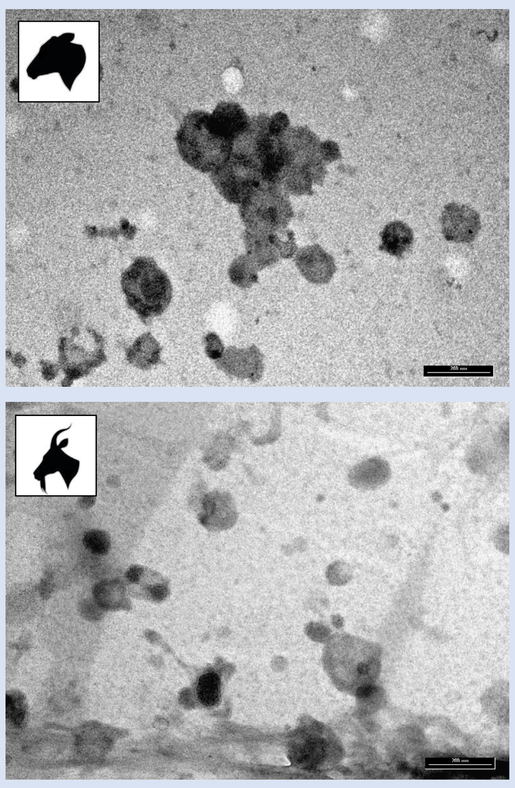


Figure 1.
Representative micrographs of isolated mEVs.
Transmission electron microscopy (TEM) was used to ascertain the presence of mEVs and to examine their morphology.
TEM observation revealed the presence of single and aggregated vesicles mainly in the range of 30-150 nm.
Scale bar: 200 nm.

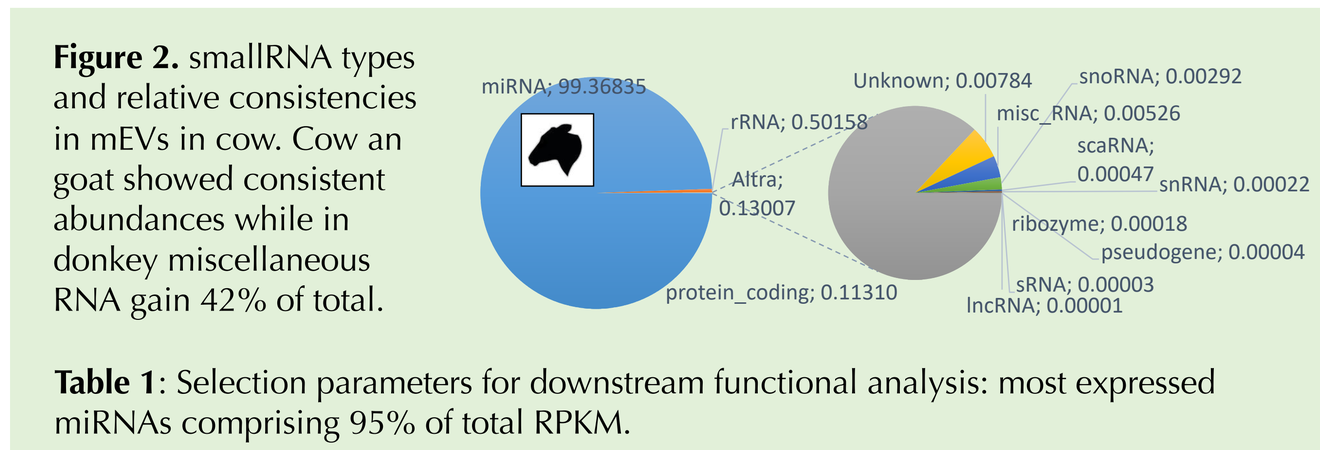
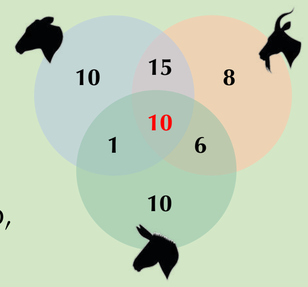


Figure 2. smallRNA types and relative consistencies in mEVs in cow. Cow an goat showed consistent abundances while in donkey miscellaneous RNA gain 42% of total.

Table 1: Selection parameters for downstream functional analysis: most expressed miRNAs comprising 95% of total RPKM.

Species	Total no. of mEV cargos miRNAs	Most expressed miRNAs	Selected Targets for functional	Selection Threshold	no. of miRNAs
cow	550	37	21		21
goat	271	39	23	56 %	22
donkey	227	27	14		15

Figure 3. miRNA shared between species.



Core: mir-151a, mir-191, mir-30b, let-7i, mir-27b, mir-30d, mir-186, mir-200b, mir-148b, mir-21

Cow Selected Targets
ABL2, ARIH1, C12orf45, CFLAR, COL12A1, COL6A3, CUX1, DMD, DNAH14, DST, FGF13, KIAA1109, LAMA1, NFIB, NKAIN3, PKHD1, RNF213, SMAD2, TTN, USH2A, ZBTB20
Goat Selected Targets
ago-01, ABL2, C12orf45, COL12A1, DMD, DNAH14, DNAH8, DST, GREM1, KIAA1109, NKAIN3, NTRK2, PDE4DIP, PKHD1, RNF213, SMAD2, TIGIT, TRANK1, TTN, USH2A, WNK1, ZBTB20, ZNF652
Donkey Selected Targets
ADGRG4, AP5M1, APOB, DYRK1A, GREM1, HEMK1, KDM5D, NTaRK3, PKHD1, SLC8A1, SMAD2, TIGIT, TTN, ZBTB20

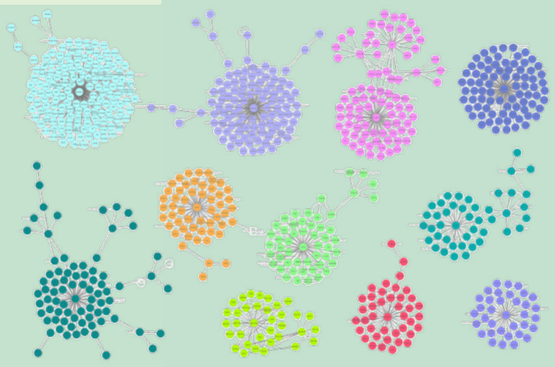
Molecular characterization

Background

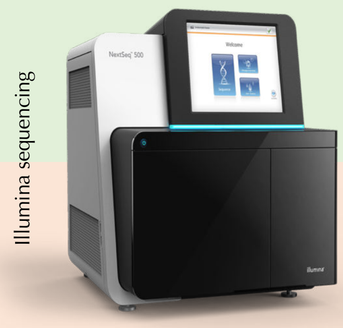
Other than being a valuable nutrition source, milk represents a sophisticated signaling system that delivers maternal messages. This property seems to be mostly mediated by Extracellular Vesicles (EVs).
Milk is among the most promising scalable and reliable source of EVs.
EVs contain different RNAs and proteins from their parental cells but have also an evolutionarily conserved set of molecules responsible for functional activities and involved in immunomodulation via the functional transfer of miRNAs, mRNAs and other constituents between immune cells.

Our **aim** is to characterize the **molecular content** of cow, donkey and goat **milk EVs (mEVs)** through RNA and metabolites **omic analysis** in view of prospective **applications as a nutraceutical in inflammatory conditions**.

Figure 4. Different clusters originated from a Protein-Protein Interaction Network (PPI) of target genes based on the number and type of connections between the nodes.



Gene Ontology analysis [FDR < 0.05] revealed enrichment for DNA methylation and histones methylation and acetylation emerged for the **cow**.
Protein formation and maturation and cellular component organization for **goat**.
Donkey seemed to comprise and strengthen all these functional messages.



smallRNA library

Quality control & Trimming → Mapping on miRBase 22 → Unmapped → Mapping on genome → RPKM normalized count matrices → miRNAs & Targets from miRWalk → Functional analysis on miRNA targets

Quality control & Trimming → Mapping on genome → RPKM normalized count matrices → 10,000 Transcripts → Functional Analysis on 10% most expressed

mRNA library

mEV mRNA cargos comparison and functional analysis on two sets of genes:
- One-to-one orthologous genes
- Species specific genes

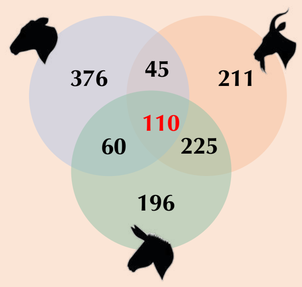
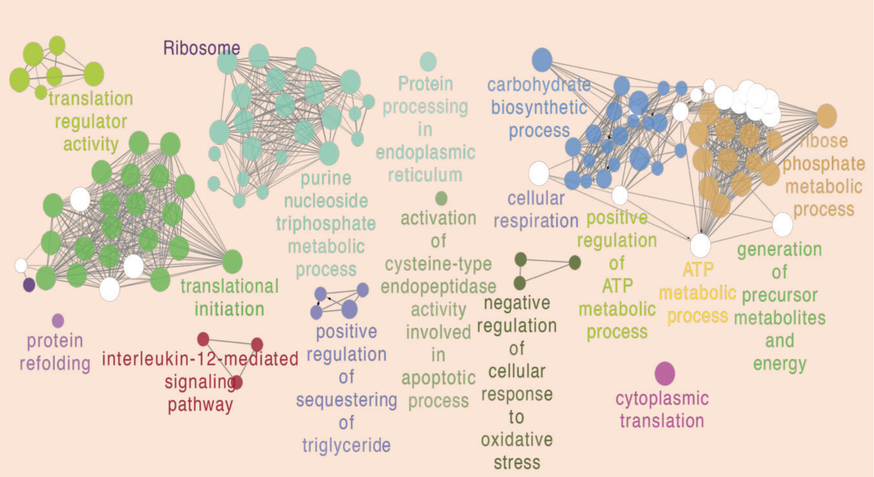


Figure 5. Orthologous genes showing the number of those shared between the species [10% most expressed].
Donkey and goat appear to be the most similar species.

Figure 6.
Protein formation, regulation to oxidative stress and IL12-mediated signaling pathway were the enriched GO terms for the **110 core** genes, pointing to the involvement of innate and acquired immunity.
Results are confirmed for genes shared by two species at the time, where terms related to energy metabolism also emerged.
Donkey and goat mEVs displayed additional terms relative to the immune system and amino acid metabolism.



Metabolomics

These results are in accordance with our previous metabolomic analysis where common pathways among the three species involving metabolites with immunomodulating effects were identified, such as arginine, asparagine, glutathione and lysine.
(DOI: 10.3390/nu12102908)

Conclusions

- mEV cargo is enriched in key mRNAs, miRNAs and metabolites relevant for immune and inflammatory response regulation and biological processes involved in cell homeostasis.
- These results are prodromal for the evaluation of mEVs' anti-inflammatory and immunomodulating activity on *in vitro* and *in vivo* models of inflammatory-based diseases.