Plant molecules as a sustainable tool to control parasitic diseases and mastitis in livestock - Oiano-Neto J., Chagas A.C.S., Chapaval L.

Embrapa Southeast Livestock, São Carlos, SP
*poster presenter: lea@cppse.embrapa.br

Parasitic diseases caused by tick *Rhipicephalus* (*Boophilus*) *microplus*, gastrointestinal nematode (*Haemonchus contortus*) and *Staphylococcus aureus* (mastitis pathogen) are one of the main barriers to develop livestock in large scale. For many years, the indiscriminate use of veterinary drugs as carrapaticides, anthelmintics and antibiotics leaded to resistance increase by parasites. As a direct consequence of this practice, farmers have increased applications doses, contributing to the contamination of meat, milk and environment. Nevertheless, secondary metabolism of plants is known to produce many molecules with a sophisticated chemical architecture and a great variety of functional groups. These molecules have been used as the starting point in some strategic research areas as pharmacology, biochemistry, ethonobotany for the development of new drugs. The main goal of this project is the prospection of active plant molecules from species belonging to Cerrado and Mata Atlântica biomes to control cattle diseases. Some *in vitro* bioassays will be applied in this study to detect anti-tick activity (*Larval Immersion Test*, *Larval Packet Test*), anthelmintic activity (*Egg Hatching Test*), as well antibacterial activity against *Staphylococcus aureus* (*Agar Diffusion Methods*, *Minimum Inhibitory Concentration*). Additionally, chromatographic and spectroscopic techniques will be used to isolate and identify the active metabolites. This integrated overview of Phytochemistry, Microbiology and Veterinary can result in a more rational way to control livestock diseases, resulting in the manufacturing of much more healthful products. Furthermore, development of new drugs using molecules derived from biodiversity is a strong argument to promote the conservation of natural sources mainly in underdeveloped countries.

Key-words: cattle diseases, plant metabolites, parasites

Embrapa project number: 03.11.01.023.00.00

PLANT MOLECULES AS A SUSTAINABLE TOOL TO CONTROL PARASITIC DISEASES AND MASTITIS IN LIVESTOCK

JOÃO OIANO-NETO[1*], ANA CAROLINA DE SOUZA CHAGAS[1], LEA CHAPAVAL[1]

[1] EMBRAPA SOUTHEAST LIVESTOCK, RODOVIA WASHINGTON LUIZ KM 234, FAZENDA CANCHIN, CAIXA POSTAL 339, CEP: 13.560-970, SÃO CARLOS - SP.
E-MAIL: DIANO@CPPSE.EMBRAPA.BR

KEY-WORDS: CATTLE DISEASES, PLANT METABOLITES, PARASITES, PHYTOCHEMISTRY. **EMBRAPA PROJECT NUMBER:** 03.11.01.023.00.00

INTRODUCTION

Parasitic diseases caused by tick *Rhipicephalus (Boophilus) microplus*, gastrointestinal nematode (*Haemonchus contortus*) and *Staphylococcus aureus* (mastitis pathogen) are one of the main barriers to develop livestock in large scale. For many years, the indiscriminate use of veterinary drugs as carrapaticides, anthelmintics and antibiotics leaded to resistance increase by parasites. As a direct consequence of this practice, farmers have increased applications doses, contributing to the contamination of meat, milk and environment. Nevertheless, secondary metabolism of plants is known to produce many molecules with a sophisticated chemical architecture and a great variety of functional groups. These molecules have been used as the starting point in some strategic research areas as pharmacology, biochemistry, ethonobotany for the development of new drugs. The main goal of this project is the prospection of active plant molecules from species belonging to Cerrado and Mata Atlântica biomes to control cattle diseases. Some in vitro bioassays will be applied in this study to detect anti-tick activity (*Larval Immersion Test, Larval Packet Test*), anthelmintic activity (*Egg Hatching Test*), as well antibacterial activity against *Staphylococcus aureus* (*Agar Diffusion Methods, Minimum Inhibitory Concentration*). Additionally, chromatographic and spectroscopic techniques will be used to isolate and identify the active metabolites. This integrated overview of Phytochemistry, Microbiology and Veterinary can result in a more rational way to control livestock diseases, resulting in the manufacturing of much more healthful products. Furthermore, development of new drugs using molecules derived from biodiversity is a strong argument to promote the conservation of natural sources mainly in underdeveloped countries.

FIGURE 1. Engorged female larvae of *Rhipicephalus (Boophilus) microplus* (**A**), heifer infested by ticks (**B**) and first stage free-living larvae of *Haemonchus contortus* shortly after hatching from the egg (**C**).

FIGURE 2. Colony of *Staphylococcus aureus*, pathogenic agent of mastitis (**A**), acute gangrenous staphylococcal mastitis (**B**) and characteristic reddish-brown secretion of gangrenous mastitis (**C**).

METHODOLOGY

FIGURE 3. Methodologies for detection and isolation of pure compounds from plant crude extracts.

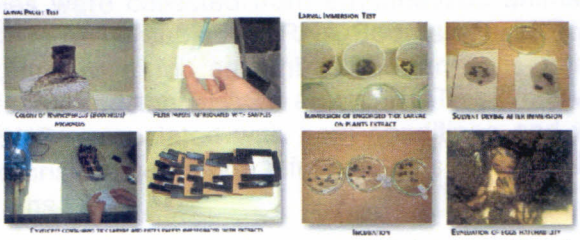
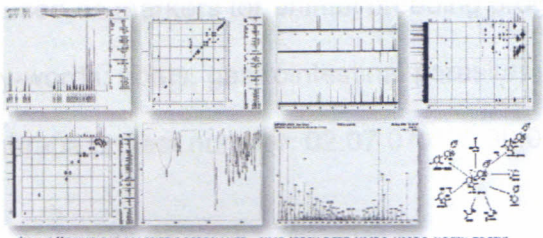


FIGURE 4. *In vitro* bioassays (*Larval Packet Test* and *Larval Immersion Test*) for measurement of anti-tick activity of crude plant extracts and pure secondary metabolites against *Rhipicephalus (Boophilus) microplus*.



^{1}H and ^{13}C NUCLEAR MAGNETIC RESONANCE – NMR (COSY, DEPT, HMBC, HMQC, NOESY, TOCSY), INFRARED SPECTROPHOTOMETRY (IR) and MASS SPECTROMETRY (MS)

FIGURE 7. Spectrometric techniques (*Nuclear Magnetic Resonance, Mass Spectrometry and Infrared Spectrophotometry*) used for structural determination of plant bioactive secondary metabolites.

FIGURE 5. *In vitro* bioassay (*Egg Hatching Test*) for measurement of anthelmintic activity of crude plant extracts and pure secondary metabolites against *Haemonchus contortus*.

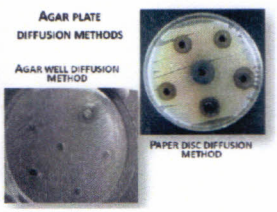


FIGURE 6. Evaluation of antibacterial activity of crude extracts and pure compounds from plant origin against *Staphylococcus aureus* by agar diffusion methods

PERSPECTIVES

(1) Contribute to the increase of scientific knowledge about the chemical composition of Brazilian plant species with potential application in controlling parasitic diseases of economic importance for the development of large-scale farming; (2) Contribute to the development of a more sustainable livestock with lower environmental impact, suggesting alternative methods of control of parasites and pathogens, providing a cost reduction with the prevention of such diseases, especially for low-income producers; (3) Contribute to reduce the growing use of veterinary products, decreasing the concentration of residues of these drugs in meat and milk, thereby increasing the nutritional quality of these foods; (4) Establish an information's set about the chemical profile of active metabolites (acaricides, anthelmintics and bactericides) of plant origin and thus generate subsidies to guide future studies on structural modification, structure-activity relationships and activity evaluation *in vivo*.

BIBLIOGRAPHY

1. BISSINGER, B. W.; ROE, R. M. Tick repellents: past, present and future. ***Pesticide Biochemistry and Physiology***. 96: 63-79, 2010.
2. COLES, G. C. Anthelmintic resistance - looking to the future – a UK perspective. ***Research in Veterinary Science***. 78: 99-108, 2005.
3. COLES, G. C.; BAUER, C.; BORGSTEEDE, F. H. M.; GEERTS, S.; KLEI, T. R.; TAYLOR, M. A.; WALLER, P. J. World Association for the Advancement of Veterinary Parasitology (WAAVP). Methods for the detection of anthelmintic resistance in nematodes of veterinary importance. ***Veterinary Parasitology***. 44: 35-44, 1992.
4. DRUMMOND, R. O.; ERNEST, S. E.; TREVINO, J. L.; GLADNEY, W. J.; GRAHAM, O. H. *Boophilus annulatus* and *B. microplus*: laboratory tests of insecticides. ***Journal of Economics Entomology***. 66(1): 130-133, 1973.
5. FAO PLANT PROTECTION BULLETIN. Recommended methods for the detection and measurement of resistance of agricultural pests to pesticides: tentative method for larvae of cattle tick *Boophilus spp*. **FAO METHOD**. 19(7): 15-18, 1971.
6. GAVIN, J. Microbiological Process Report: Analytical Microbiology - II. The Diffusion Methods. ***Applied Microbiology***. 5: 25-33, 1957.
7. GIBBONS, S. Anti-staphylococcal plant natural products. ***Natural Product Reports***. 21(2): 263-277, 2004.
8. GURIB-FAKIM, A. Medicinal plants: traditions of yesterday and drugs of tomorrow. ***Molecular Aspects of Medicine***. 27: 1-93, 2006.
9. KINGSTON, D. G. I. Modern natural products drug discovery and its relevance to biodiversity conservation. ***Journal of Natural Products***. 74: 496-511, 2011.
10. LIMA, M. R. F.; EULÁLIA C. P. AZEVEDO XIMENES, E. C. P. A.; LUNA, J. S.; SANT'ANA, A. E. G. The antibiotic activity of some Brazilian medicinal plants. ***Brazilian Journal of Pharmacognosy***. 16(3): 300-306, 2006.