USO DE BIOTERÁPICO DE *MYCOPLASMA* SPP EM REBANHO BOVINO LEITEIRO

THE USE OF *MYCOPLASMA* SPP BIOTHERAPIC IN A DAIRY CATTLE HERD

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ABSTRACT

This experiment has been conducted in an organic milk production farm, where there have been breeding problems and sporadic outbreaks of respiratory disease.
A treatment with Hydrastis has been made based on the repertorization of the epidemic nature of the respiratory disease outbursts, the results of which, however, were just palliative, with recurrence of the symptoms.
In researching into a possible etiologic agent, a Mycoplasma spp was found, based on a culture of which a biotherapic remedy dynamized at 30CH was performed. During this study, 10 drops of the Mycoplasma spp was diluted in the water drunk by the animals over a 6-month period.
A week after starting medication, the respiratory symptoms gradually lessened, with concurrent enhancement of the breeding capacity, represented by a higher AI (artificial insemination) ratio from 5 to 2.5 doses per animal, and 20% of animals getting pregnant at the first AI.
The monitoring process consisted of 4 series of bacteriological exams (based on vaginal and nasal mucus swabs) performed in July, October, January and March 2004, all with negative results.

Key words: bovine, biotherapic, treatment, *Mycoplasma*, homeopathy

INTRODUCTION

This experiment has been conducted at an organic milk production farm, formerly Fazenda da Conceição, in the Vale do Rio Paraíba, Municipality of Lorena, SP, where Instituto Oikos de Agroecologia (a Civil Public Interest Society´s Organization) is headquartered.

The farm owns a 90-head herd of Holstein, of which 50 are adult females. Of these, 36 are lactating cows, producing 500 liters per day in average.

After the heat repetition and decreased respiratory function episodes affecting the herd were diagnosed as caused by *Mycoplasma* spp., a specific homeopathic treatment was sought.

Organic Handling

With the organic production increasing at a fast pace in the world, it could not be different in Latin American countries. In Brazil, which currently ranks second in organic areas among Latin American countries, it is estimated that some 100 thousand ha. are being cultivated by approximately 4,500 organic production units. The value of the

Brazilian organic production is estimated at "between US$120 and US$200 million each year. [11]"

According to the organic agriculture principles, animal-related activities must be as integrated as possible with vegetable production, with a view to optmizing nutrient recycling (animal excrements, vegetable biomass), reducing dependence on external input (feed) and enabling all direct and indirect benefits arising from such integration.

As regards veterinary treatment, the main objective of organic breeding methods is to prevent disease. Whenever a disease appears, it is mandatory to find the cause and prevent recurrence thereof, by changing handling techniques. However, given the need for treatment, one has to look to therapeutic methods such as homeopathy and phytotherapy, among others.

In an attempt to comply with organic production concepts, a homeopathic alternative was sought to solve reproductive and respiratory problems that have stricken the herd.

***Mycoplasma* spp.**

Mycoplasma, of the *Mollicutes* class, is characterized by comprising smaller and simpler free life microrganisms already known, and especially by the absence of cellular membrane. Given their genome´s minute size, they have limited biosynthesis capacity. The absence of cellular membrane makes them suscetible to osmotic shock, but resistant to beta-lactam antibiotics such as penicilins.

Regardless of their genetic simplicity as the only bacteria on the IEO (International Epizooic Diseases Organization), a list of animal diseases of mandatory reporting, the components of the *Mollicutes* class are deemed to be pathogens of utmost importance.

Found all over the world, the mycoplasmas are very important in every cattle raising areas.

Vaccines provide some protection against diseases and reduce economic losses, but fail to prevent colonization and microorganism elimination, with disease outbursts still occurring with unacceptable frequency.

The mycoplasmas have been isolated and are frequently considered as the cause of diseases in bovine respiratory tract, genitalia, mamal glands, joints and eyes. "Although different mycoplasma species have been isolated therefrom, only three diseases surely have micoplasmas as their primary etiologic agents, namely Contagious Bovin Pleuropneumonia (*Mycoplasma mycoides* subsp. *mycoides*), mastitis and arthritis caused by *M. bovigenitalium e M bovis* [12]".

Many mycoplasma species may be part of the flora that inhabit the mucous membrane lining the upper respiratory duct, the mouth and the genitalia. According to the pathogenicity and location, the *Mollicutes* will be involved in respiratory and genital-urinary diseases. Likewise other infectious agents, the host/parasite relationship will depend on the host´s resilience, the infecting dose and the agent´s, as well as the environmental virulence. Accordingly, no wonder that where the resistance of a single individual or a group of animals is low, a subclinical micoplasmosis may develop, with clinical signs at different places in the body.

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That is why mycoplasmas may be involved in mastitis, arthritis, epididymitis, orchites, urethritis, conjunctivitis and meningitis, to say nothing of the classical above mentioned syndromes.

Isopathy and Biotherapic Products

The term Isopathy, of Greek origin (iso = equal; and pathos = suffering) refers to the treatment of disease by means of the causal agent or a product of the same disease one intends to cure.

In a broad sense, isopathy is the method of treating disease through its causal agents handled in accordance with homeopathic techniques.

Like Homeopathy, Isopathy uses dilluted and dynamized medications, which in the latter case are called biotherapic products.

These products are prepared based on cultures of bacteria, fungi, viruses, pathological secretions or excretions, acarids, worms etc., of both animal and vegetable origin.

Currently, the use of biotherapic products relies on research studies conducted by the veterinarian Guillermo Lux. In a paper published in 1833, “Isopatia de las enfermidades contagiosas”, Lux demonstrated the treatment of bovine pest and carbuncle, using the blood of carbuncle-infected animals or the nasal secretion of pest-stricken animals, all dynamized at 30CH.

Over the years, this method has proven effective in the treatment of allergies and chronic or recurrent infections. Biotherapic products are prescribed based on a patient´s clinical history and other diagnostic methods such as sorology or isolation of etiologic agents.

The reason for opting for Mycoplasma biotherapies was the lack of a satisfactory allopathic treatment, the impossibility of using antibiotics during organic handling routines, and the inneficiency of treatment with a homeopathic medicine (Hydrastis), chosen according to the epidemic nature of the herd´s disease.

Material and Methods

Before being organically handled, this herd had low fertility levels, continuing use of hormones being necessary for females to be in heat and become pregnant.

By simply analyzing the ratio of artificial insemination (A.I) to pregnancies obtained (since at that time a bull was kept at the farm for breeding cows which repeated heat more than three times) we arrived at 5 A.I. per pregnant cow.

Hormonal treatment with gonadotropins and prostaglandins (Ciosin®, Fertagyl®, Conceptal®, Profertil®) was frequently used, too.

After changing to the organic handling, there came the need for adjusting the sanitary, nutritional and reproductive handling of animals, since the concentrated volume in the diet and the therapeutic protocols used were incompatible with the organic production requirements.

Given the high heat repetition indices, examinations were made in search of infectious diseases that might be affecting the animals.

In 2002, 23 animals were tested for IBR and Neospora, of which four samples were IBR positive and one Neospora positive.

However, the findings did not justify the heat repetition observed.

Irrespective of the reproduction picture, the animals began to have respiratory malfunction, which affected the whole herd (all categories of animals) with 50% morbidity. The symptoms were as follows: great quantity of yellowish nasal secretion, reduced apetite, decreased activitity, but without lung stertor and fever.

The bovine respiratory system is continuously exposed to potentially pathogenic microorganisms. However, most of the time the animals remain healthy due to their pulmonary defenses.

"Mycoplasma spp., *Ureaplasma diversum, Pasteurella haemolytica* (*Mannheimia haemolytica), P. multocida, Haemophilus sommus (Histophilus somn), sincicial respiratory virus, parainfluenza 3 virus,* and one bovine herpesvirus 1 are microorganisms possibly found, often in synergy [3]". Consequently, laboratory tests were performed in an attempt at diagnosing the disease affecting the herd.

Five nasal mucus samples of 10% of animals affected were subjected to bacteriological tests.

Bacterial isolation

Five nasal mucus samples collected by nasal swab were sent to the Laboratório de Doenças Bacterianas da Reprodução do Instituto Biológico, in São Paulo (Instituto Biológico/São Paulo Bacterial Reproduction Diseases Laboratory). The samples were kept in transport medium (A_3XB) under refrigeration until the laboratory processes began. In order to arrive at a *Mycoplasma* spp. and/or *Ureaplasma diversum* diagnosis, the samples were processed as follows.

The vaginal and nasal mucus, and the semen samples, were plated in specific *Mycoplasma* and *Ureaplasma* culture media, M (Hayflick) and U agar, broth M and U, according to Ruhnke and Rosendal[20].

The M and U agar were rubbed with *swab* and subsequently, using the transport medium containing mucus, 0.3 mL of the suspension was used for three decimal dillutions in M and U broth, totalling two plates and six tubes per clinical sample processed, for subsequent reading. The agar plates were incubated in a microaerophilic jar for fifteen days at 37°C, in a 95% N_2 + 5% CO_2 (WHITE-MARTINS) atmosphere of residual O_2, after removal of air from the interior of the jar (-600 mmHg) using a vacuum pump for this purpose. The broth were incubated for up to 15 days at 37°C, in aerobiosis.

The plates and tubes were observed every day for 15 days in a row. The agar plates were read using a stereoscopic 40 X magnifying glass.

The *Mycoplasma* and *Ureaplasma* growth was characterized base don typical colonial morphology, where grown on agar. The growth in broth was observed through changes in colouring thereof (due to changes in pH, the yellow broth turns to pinkish) and/or through sub-cultivation (broth ⇨ agar) for confirming bacterial growth.

Concurrently, other bacterial agents possibly involved in clinical cases were researched. To this end the clinical material was cultivated in Brucela Blood Agar[4].

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Table 1 shows the results of laboratory processes.

Table 1. Bacteriologic tests performed in April 2003

Animals	Tests	Test Date	Type of material	Diagnostic Method	Results	Symptoms
05	*Haemophilus*	04/26/2003	Nasal mucus	Isolation in culture medium	Negative	Reproductive, Respiratory
05	HVB	04/26/2003	Nasal mucus	Isolation in cellular culture	Negative	Same as above
05	*Ureaplasma*	04/26/2003	Nasal mucus	Isolation in specific culture medium	Negative	Same as above
05	*Mycoplasma*	04/26/2003	Nasal mucus	Isolation	4 positive	Same as above

Based on the *Mycoplasma* diagnosis, we began to consider using biotherapic products for treatment of this disease, which cannot be satisfactorilly treated with the conventional antimicrobiological therapies.

Biotherapic Product Manufacturing

The medicine was prepared in accordance with the ABFH (Associação Brasileira de Farmacêuticos Homeopatas, Brazilian Homeopathic Pharmacists Association) Technical Standards Manual and the rules and methods set at the Brazilian Homeopathic Pharmacopeia. The dynamization method used was the Hahnemannian method. Inert input used: hydroalcoholic solution at 70%. Scale: centesimal

The method consists of serial dillutions, each of which is followed by sucussions, a component of dynamization. Each step corresponds to a dillution followed by dynamization, the next dillution being always 1% of the previous one.

The medicine was prepared out of a culture of dead *Mycoplasma* spp. cells at 1% in a 10 mL alcoholic solution. The first five steps were performed at the Instituto Biológico/São Paulo laboratory, on a laminar flow, the shaking being made with the aid of a vortex. The potencies 4CH and 5CH were taken to the HN Cristiano/Sao Paulo laboratory and raised up to 30CH aided by a mechanic agitator.

Flasks at 30CH 100% were prepared (dynamized solution) and sent for dillution and use in the herd.

Use of Medicine

The animals (50% of the herd) suffering from respiratory symptoms were treated with Hydrastis 12 CH, according to repertorization based on the epidemic nature of the disease (nasal and post-nasal yellowish, viscous secretions were the most characteristic

signs, which led us to think of Hydrastis and Kali bichromicum. However, as the only symptom was reduced appetite, with no pain whatsoever, Hydrastis was chosen).

In July 2003 there was a new outburst of respiratory disease, which began with the lactating cows, again treated with Hydrastis (12CH). However, the outburst spread to the whole herd.

Using the bacterial culture as a basis, a "biotherapic product was prepared with dynamization of up to 30CH" [15], who does not recommend the use of biotherapic products of less than 30CH.

On July 20, 2003 a new treatment began by adding this biotherapic product to the herd´s drinking water: 10 drops/water hod/day. All hods, which had automatic water replacement, were used in the treatment.

The whole herd was treated for six months.

Monitoring

The process monitoring comprised four sequences of examinations in July and October 2003, and in January and March 2004.

On July 21, 2003, five more samples were sent for testing, two of vaginal mucus and three of nasal mucus (in selecting the animals, priority was given to those with fertility problems). The results of all these samples were negative.

On October 8, 2003 seven further samples of secretions and semen of three bulls used for A.I. at the farm also had negative results.

On January 18, 2004 the results of five samples of nasal secretion and five paired samples of vaginal secretion (nasal and vaginal secretion of a single animal) sent for testing were negative.

On February 29, 2004, a cow three months pregnant had an abortion. After that, samples of placenta and cervical mucus were sent to be examined, the presence of *Ureaplasma diversum* being detected through a PCR - Polymerase Chain Reaction, but not of *Mycoplasma spp*.

No bacterial isolation was feasible due to the excessive secondary contamination of the clinical material.

Table 2. Results of subsequent examinations

Samples	Tests	Test Date	Type of material	Diagnostic Method	Results	Symptoms
03	*Ureaplasma*	0721//2003	Nasal/vaginal mucus	Isolation in specific culture medium	Negative	Fertility-related, respiratory
03	*Mycoplasma*	07/212003	Nasal/vaginal mucus	Same as above	Negative	
05	*Mycoplasma*	10/08/2003	Nasal/vaginal mucus	Same as above	Negative	
05	*Ureaplasma*	10/08/2003	Nasal/vaginal mucus	Same as above	Negative	
03 semens	*Mycoplasma*	10/08/2003	Palets of semen	Same as above	Negative	
03 semens	*Ureaplasma*	08/2003	Palets of semen	Same as above	Negative	
05	*Mycoplasma*	/01/182003	Nasal/vaginal mucus	idem	Negative	
01	*Mycoplasma*	10/01/03/04	Vaginal Mucus/cotiledons	PCR	Negative	Abortion
01	*Ureaplasma*	01/03/04	Mucus/cotiledons	PCR	Positive	abortion

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FINDINGS AND CONCLUSION

After the treatment began, the symptoms decreased. 20% of production animals which had hut repetition got pregnant and the pregnancy was completed successfully. A further 20% got pregnant at the first insemination.

The effectivity of semen doses used grew by 50%.

There was no recurrence of respiratory malfunction.

The herd´s reproductive performance confirms the effectiveness of the treatment, through reversal of hut repetition and remission of respiratory symptoms. This paper signals that the prospects of effectively using biotherapic products for treating a few diseases are good, irrespective of use of antibiotics. This might render some handling aspects of organic cattle-raising feasible, while reducing treatment costs (homeopathic treatments are 70% less expensive than conventional ones, in average) and, most importantly, reversing some dormant infection and carriers individuals conditions, according to experiments in human toxoplasmosis conducted by Costa [5].

Other herds raised in different regions with different sanitary and nutritional handling methods need to be tested for better evaluation of the medicine effectiveness and the animals´ serologic and physio-pathologic behavior.

In therapeutic terms, from the moment a general practicioner begins to question their own methods and the cost-benefit relationship of conventional treatments, they are seeking homeopathy, a way to open a new door for themselves and their patients/clients.

And when we, ourselves, as homeopaths question our own concepts and search for new options (the use of Isotherapy, for example) we are pursuing the same goal. This search involves changes in the doctor-patient relationship, the approach to anamnesis, semiology, clinic and diagnosis. “This does not mean that diseases themselves have changed, but the value assigned to each piece of this puzzle represented by clinical and therapeutic procedures, as well as disease prevention” [2].

Accordingly, despite Hahnemann´s condemnation of Isopathy (Organon, paragraph 56), we follow Alves´s considerations [1], quoted by Benez[2].

What would be the purpose of curing an infection separately from the organism as a whole? Any toxic agent interferes with a patient´s disease, making it difficult to identify a single constitutional remedy constitutional remedy for him/her. By freeing the organism from these endogenous or exogenous toxins only pure symptoms will be left, thus facilitating identification of the constitutional remedy. This “cleaning” of effects of bacteria can be done through Isopathy.

Still on the Organon paragraph referred to above, Vijnovsky [23] comments that “it is very common for a homeopath to nosodes, nowadays, often as a very useful therapeutic

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means to cure disorders or diseases obviously originating from an infectious disease. In these cases, using a nosodes is a legitimate and effective resource, especially where a "simillimum" does not seem to be effective".

Another possible treatment is the association of a medicine of epidemic nature with a biotherapic product, in endemic cases involving infectious, contagious diseases, when the causal agent and all the symptoms are known.

In sum, we do find that Isopathy is worth being studied in the light of modern technologies (serological, hemathological and microbiological) as a tool for controlling the effects of some diseases on the population.

In this sense, veterinary research has a lot to contribute, since with animals it is possible to generate more data on the immunity of humans, both individuals and populations, as well as on the effects of a medicine when faced with a challenge. (inoculation of the agent or experimentally-induced endemic diseases.

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