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A vacinação obrigatória de fêmeas de três a oito meses é uma das principais ações do Programa Nacional de Controle e Erradicação da Brucelose e Tuberculose (PNCEBT) no Brasil. O Instituto Mineiro de Agropecuária (IMA), com o apoio da Vallée, executa o Projeto de Apoio à Saúde Agropecuária (Pasa) desde 2002. Seu princípio é desenvolver metodologias educativas para promover a sanidade animal e consciência para sustentabilidade e saúde. O Pasa tem inovado na inclusão social, ocupação e renda de pequenos agricultores, fixando o homem no campo. Os Agentes de Saúde Agropecuária (ASA) realizam vacinações para buscar os índices acima de 80% recomendados pelo Ministério da Agricultura (Mapa). Com o objetivo de avaliar a relação entre os índices de vacinação e a distribuição de animais nos rebanhos e atuação do Pasa, pois a maior dispersão de animais em inúmeros rebanhos dificulta a ação de vacinação, foram coletados dados do cadastro do IMA de 1/1/2007 a 31/12/2007. Foram estudados 77 municípios do norte do Estado de Minas Gerais, desses 11 com o Pasa em funcionamento, a mais pobre região do Estado. É caracterizada por escassez de centros urbanos e profissionais autônomos, rebanhos com baixa tecnologia e baixa qualidade da terra. O clima é quente, beirando o semiárido, com longos períodos de estiagem. As análises foram efetuadas no SPSS 18.0. Foram retirados dados considerados “outliers”. Foi realizada a análise descritiva de todas as variáveis levantadas e calculadas a correlação de Pearson e regressão linear simples e múltipla considerando-se como variável dependente a “percentagem de vacinação” e como independentes a “presença/ausência do PASA” e a “% de rebanhos com mais de nove bezerras em idade 0-12 meses”. O nível de confiança utilizado foi de 95%. A média de vacinação foi de  $69,69 \pm 19,47$  e de “percentagem de rebanhos com mais de nove bezerras” foi de  $71,88 \pm 16,47$ , com correlação de 49,2% ( $p = 0,000$ ). A cada 1% que aumenta a concentração de “rebanhos maiores de nove bezerras” há um aumento de 0,581 na “% de vacinação”. “Rebanhos que possuem Pasa” têm em média 29,27% a mais de vacinação ( $IC_{23,95-34,59}$ ;  $p = 0,000$ ). Municípios sem Pasa têm média de vacinação de  $65,50 \pm 17,75$  e com  $94,77 \pm 5,02$ . No modelo múltiplo, os incrementos médios ajustados para “com Pasa” = 29,16 e a cada “% de rebanhos maiores” = 0,58 ( $p = 0,000$ ). Concluímos que é fundamental a presença do Pasa naqueles municípios em que há maior dispersão de bezerras.

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### Contamination by mycotoxins in dairy production systems, Paraná State, Brazil\*

Contaminação por micotoxinas em sistemas de produção leiteira no Estado do Paraná, Brasil

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Aiming to identify and characterize milk and feeds contamination by mycotoxins it were monitored 96 milk farms in 2009/2010. The study was made in three regions of Paraná state, representatives of dairy production according

to report of Ipardes in 2009. Two kinds of data were collected: samples of feedstuff offered to herd, milk, water and in the other hand, production systems data, collected by a guided interview and questionnaire. Toxicology analysis was made according to methodology described by Soares e Rodriguez-Amaya for feedstuff and by ELISA immunoassay kit for aflatoxin M<sub>1</sub> – AFM<sub>1</sub> in milk. Comparison for regions and seasonal variation of contamination was analyzed by Generalized Linear Models – GLM. Data of milk contamination by mycotoxins and their sources were analyzed under path analysis method. Information collected by questionnaire was related to: storage process, type of feedstuff and supply period of these feeds. Contamination prevalence was 29.2% of all feed samples for mycotoxins. The main contamination by mycotoxins was related to corn and these byproducts ( $p < 0.05$ ), especially commercial concentrates. On the other hand, aflatoxins were predominant ( $p < 0.05$ ) over the other mycotoxins metabolites, overall the aflatoxin B<sub>1</sub> – AFB<sub>1</sub>. Milk contamination measured for AFM<sub>1</sub> presented seasonal variation ( $p < 0.05$ ), lower concentrations in rain periods and higher in dry periods. This can be explained for the increase of concentrates, silage and hay supply in dry period, due to restriction in the forage mass production. There was no effect for regions concerning to AFM<sub>1</sub> concentrations, but the range of variation was large, 0.12 to 1.20 µg/L. This shows that the Dairy Production Systems – DPS in farms are widely heterogeneous in terms of practices and this interference in contamination of dairy production by mycotoxins. The main source of milk contamination (AFM<sub>1</sub>) was the presence of metabolites of aflatoxins in feedstuff, independent of the feed source. Corn and those byproducts have weak direct effect in AFM<sub>1</sub> concentrations. This means that the contamination by aflatoxins is generalized among the feed sources and there is no one main source for contamination in analyzed feeds. The major direct effect on AFM<sub>1</sub> (0.51) was observed for AFB<sub>1</sub>, which is reinforced by information available in literature. AFB<sub>1</sub> is the major metabolic precursor of AFM<sub>1</sub>, in mammal's organisms. We conclude that aflatoxins are the main mycotoxins contaminants in feeds and it is widely spread in all studied regions. There is a seasonal variation in those concentrations in feed, consequently in AFM<sub>1</sub> milk levels.

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### Detection of pathogens of *Apis mellifera* L. (Hymenoptera, Apidae) in honey with multiplex PCR and its use in Brazilian samples\*

Detecção de patógenos de *Apis mellifera* L. (Hymenoptera, Apidae) em mel com PCR multiplex e seu uso em amostras brasileiras

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Several pathogens attack the bees *Apis mellifera* L. (Hymenoptera: Apidae) around the world, such as the bacteria *Paenibacillus larvae* and the fungi *Ascosphaera apis*, *Nosema apis* and *Nosema ceranae*. Their distributions in some parts of the world, such as Brazil, are not fully known not only because

of the difficulty of collecting samples such as in our large country, but also because of the time and the cost of diagnosis techniques involved. The analyze of the presence of the spores of honeybee pathogens in honey has shown to be a good strategy for epidemiological studies and early detection before the expression of symptoms in the colony. Therefore it is important the standardization of techniques for rapid diagnosis to facilitate the safe performance of the epidemiological surveys, and controlling the spread of these microorganisms. Here, we it was standardized a multiplex PCR technique for simultaneous detection of four pathogens of *A. mellifera*: *A. apis*, *N. apis*, *N. ceranae* and *P. larvae* in honey. This technique was used in honey samples from some Brazilian states. Sterile honey samples (20 mL) were artificially contaminated with all selected pathogens. These positive samples were diluted in 30 mL sterile water followed by centrifugation. DNA from pellet was extracted using a commercial kit. A Multiplex PCR was standardized using specific primers and a common melting temperature. Recommendations of national legislation were used for preparation of honey solutions submitted to the developed technique. Also it were prepared samples of honey collected from brood area, extracted honey from supers by beekeepers and acquired from different commercial establishments used to validate the multiplex PCR, as well as to conduct a preliminary assessment of the distribution of pathogens (*A. apis*, *N. apis*, *N. ceranae* and *P. larvae*). The standard technique of this study was effective for diagnosing of the four pathogens in honey of *A. mellifera*: *A. apis*, *N. apis*, *N. ceranae* and *P. larvae*. Primers used in both PCR reactions (monospecific and multiplex) for DNA amplification of the target pathogens were precise and sensitive, resulting in products of expected sizes. The formation of nonspecific fragments and/or other artificial PCR products was not observed in multiplex PCR reactions. Thus, this method was suitable for simultaneous detection of the selected pathogens of *A. mellifera* extracted from honey, and probably can be used in other hive products with minor modifications. The selected pathogens were not found in the honey samples analyzed with this multiplex PCR standardized.

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In Brazil, though the Africanized honeybees are considered resistant and tolerant to most of bee pathogens and parasites, in recent years considerable losses have been reported in some localities, especially in the Southeast and Southern states. Since the phenomenon that is affecting honeybees around the world has been observed also in Brazil (with less intensity), we proposed a research project aiming to determine possible causes for such decline or losses, and in particular to test the likelihoods of specific predicted causative agent(s) for this condition. Alternative approaches, including genetic screening can be extremely useful and can accelerate important discoveries related to the current problem. Here it was present an overview about these activities and a summary (2009 to 2011) of the situation in the two regions. No symptoms of the American Foul Brood were detected and analysis of more than 600 honey samples presented negative results, using the Brazilian official method for microbiological detection of *Paenibacillus larvae* in honey (molecular techniques confirmed such absence). *Leptomonas apis*, and *Spiroplasma apis* were also detected in São Paulo State samples. One of the main sanitary problems of the Africanized bees (beekeeper loses, sometimes, all colonies during less than one-month period) is Brazilian Sacbrood-like disease, caused by the toxic effects of the *Stryphnodendron* spp pollen in the Atlantic Forest and Savannah regions (Sac Brood Virus has not been detected in these colonies and the symptoms are similar). Nowadays, the selection for resistance is under evaluation. Other brood mortality has been also observed during the last years showing anomalous symptoms. In adult bees different type of viruses such as ABPV, DWV, BQCV, IAPV have been detected, and previously CWV, FV and CBPV. Accidents with insecticides have been related. Collapse and mortality of adult bees have been detected in sugar cane areas where neonicotinoids normally are used, but in areas without sugar cane crop too. *Nosema ceranae* is present in many apiaries, showing high prevalence. The obtained results indicated that *N. ceranae* infection seems to suppress bees immune response of *A. mellifera* due the peptides transcription of the antimicrobes abaecina and himenoptaecina. *N. apis* was detected only in some localities of Santa Catarina and Paraná. *Varroa destructor* is present throughout apiaries, but causing no apparent direct economical damages. Replacement of the queens from beehives showing symptoms has been recommended, avoiding chemotherapeutic drugs usage. There is a critical need to increase the number of technicians and laboratories dedicated to bee pathology in several regions of the country in order to have a better control of the sanitary situation in the apiaries. Pathological, epidemiological, and widespread studies must be conducted as no single pathogen seems to be predominant in declining bee colonies.

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## Honey bee health and losses in Brazil

Sanidade apícola e perdas no Brasil. Sanidade apícola e perdas de colônias no Brasil

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