TOPIC: Arthropod-borne diseases of veterinary importance
APPROACH: Vector biology and eco-epidemiology

Detection by PCR of *Culicoides insignis* Lutz (Diptera: Ceratopogonidae), the main vector of bluetongue virus (BTV) in the Neotropical region

Keywords: mosquitoes, PCR, detection, bluetongue virus, neotropical region

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Bluetongue Virus (BTV) cause a viral, non contagiosus disease that mainly affects sheep, cattle and wild and farmed ruminants causing damage to these animals and significant economic losses. It is well known in Central America and the Caribbean, and even in the Lesser Antilles, and in South America this virus has been isolated in Brazil, Argentina, Peru, Ecuador and Guyana. *Culicoides insignis* Lutz, the major BTV vector in South America, is one of the most frequent and abundant species found in Southeastern USA, the Caribbean Basin, and Central and South America, that is primarily associated with cattle farms. Accurate identification of biting midges is essential for the understanding of disease epidemiology and vector control. Morphologically, *Culicoides insignis* is placed in the *Culicoides guttatus* group, the females are easily recognized from congeners of this species group by a combination of three wing characters: the r-m crossvein is distinctly dark, the vein R_3 is dark up to the point where it turns abruptly forward to meet the costa, and by the single distal pale spot in cell M_1; other useful characters are the third palpal segment bearing an irregular sensory pit and the distal sensilla coeloconica always present on flagellomeres 1, 3, 5, 7 and 9-13, and sometimes present on flagellomeres 2, 4, 6 and 8. The male wing frequently exhibits a second pale spot at wing margin in cell M_1. In the other hand, the molecular tools applied to taxonomy provide rapid and efficient method to the identification of vector species. We designed a forward primer since a specific sequence of the ITS-1 region of *C. insignis*, which does not generate amplification

products in the other analyzed *Culicoides* species. To test the specificity of the primer in vitro we worked with the five most-abundant species captured during the fieldwork in Misiones province. Later, because of the lack of ITS-1 sequences in the species of the guttatus group or some sequence of any neotropical *Culicoides* in the database, the primer in sílico specificity was checked through alignment with other *Culicoides* sequences published in GenBank with the aid of the online BLAST tool, where the alignment was carried out according to the parameters established by default of the program. The use of molecular biology tools applied to the specific identification of species can simplify the taxonomic identification process of *Culicoides* midges and will contribute significantly in the case of cryptic species. With a specificity of 100%, this method could also be used for larval identification and epidemiological surveillance of these species.