

## **Anti-*Strongyloides venezuelensis* IgY: production, characterization and application in the immunodiagnostic of human strongyloidiasis.**

Lucas Silva de Faria<sup>1</sup>, José Eduardo Neto de Sousa<sup>1</sup>, Dayane Lorena Naves de Souza<sup>2</sup>, Raphaella Paula Ribeiro<sup>3</sup>, Isabela Pacheco Borges<sup>2</sup>; Veridiana de Melo Rodrigues Ávila<sup>2</sup>, Álvaro Ferreira Júnior<sup>3</sup>, Luiz Ricardo Goulart Filho<sup>4</sup>; Julia Maria Costa-Cruz<sup>1</sup>.

1- Laboratório de Diagnóstico de Parasitoses, Universidade Federal de Uberlândia, Uberlândia, MG, Brasil.

2- Laboratório de Bioquímica e Toxina de Animais, Universidade Federal de Uberlândia, Uberlândia, MG, Brasil.

3- Programa de Pós-Graduação em Sanidade e Produção Animal nos Trópicos, Uberaba, MG, Brasil.

4- Laboratório de Nanobiotecnologia, Universidade Federal de Uberlândia, Uberlândia, MG, Brasil.

[lucassilvafaria@hotmail.com](mailto:lucassilvafaria@hotmail.com)

In view of the magnitude of strongyloidiasis, rapid and effective diagnosis is extremely important. In this way, the development of new diagnostic tools is essential to help avoid chronic cases and hyperinfections in the course of this geohelminthiasis. IgY technology is a great alternative for the production of antibodies with a high degree of specificity and rentability. This study aimed the production and fractionation of IgY antibodies from egg yolks of hens immunized with total antigenic extracts of infectious filariform larvae (iL3) and parthenogenetic females (pF) of *Strongyloides venezuelensis*. The two types (anti-iL3 and anti-pF IgY) of selected antibodies were evaluated in the recognition of the parasitic protein complex and in the serological diagnosis of human strongyloidiasis. The laying hens were divided into three groups with two animals each: I) filariform larvae (iL3); II) parthenogenetic females (pF); III) phosphate-buffered saline. Six immunizations were performed, the first with complete Freund's adjuvant and the others with Freund's incomplete adjuvant with a 15-day interval and followed up for 13 weeks. Eggs and blood for serum collection were weekly collected to monitor the production of specific antibodies. The egg yolks went through three purification steps: delipidation; precipitation of proteins with 20% ammonium sulfate; fractionation with *HiTrap IgY Purification* affinity column, in *Akta prime plus* complete chromatography system. The fractionation and specificity of the antibodies were confirmed by Dot-blot, 12% SDS-PAGE under reduction conditions, ELISA, ELISA avidity, Immunoblotting and Immunofluorescence antibody test (IFAT). The fractionated IgY antibodies were used for the detection of immune complexes in serum of patients with confirmed strongyloidiasis, other parasitic diseases and healthy individuals by ELISA. Proteins from the heavy (65kDa) and light (20kDa) chains from the IgY molecule were visualized on 12% SDS-PAGE. The specificity to the parasite antigens (iL3 and pF) was confirmed by the recognition of protein bands from the total extracts (Immunoblotting) and by IFAT in histological sections of *S. venezuelensis*. IgY antibodies showed high levels of avidity ranging from 72.5% to 95.4%. The detection of immune complexes in serum samples showed diagnostic values for anti-iL3 IgY and anti-pF IgY antibodies respectively: 87.2% and 92.3% sensitivity; 88.5% and 90.6% specificity. It was concluded that IgY technology can be an excellent tool for the study of strongyloidiasis and with possibilities for application as a serological diagnostic method and for therapeutics of the disease.

**Keywords:** Immunoglobulin Y; immunodiagnostic; strongyloidiasis.

**Support:** CAPES; FAPEMIG, CNPq.