Production of polyclonal anti *Trypanosoma evansi* and evaluation by fluorescence microscopy

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*Trypanosoma evansi* is a flagellate hemoparasite of veterinary importance, which affects mainly horses, causing a condition called "Surra" or "Mal das cadeiras." For the present study, we obtained the parasites from Wistar rats infected with *T. evansi* obtained from cryopreserved isolated PVL-2005 and maintained in high parasitemia in vivo. Intracardiac puncture was performed and blood was separated by Percoll ® gradient and parasites were purified on DEAE-cellulose column. We perform two methods for the production of anti-*T. evansi* serum: In the first one we used a total protein extract, obtained using lyse solution (2.2 M thiourea, 7.7 M urea, 4.4% CHAPS) and protease inhibitor cocktail. The second method used the purified parasites fixed in 4% formaldehyde at a concentration of 35.5 x 10⁶ parasites / ml. 10 mg of total protein extract or 250 µL fixed parasites were inoculated in female rats (*Rattus norvegicus*), every 15 days during two months, using Freud’s complete adjuvant in first inoculation and the incomplete adjuvant subsequently. After the period, the blood was collected and the hyperimmune serum was tested by indirect immunofluorescence microscopy (IFA) against the purified fixed protozoa at a concentration of five parasites / field. The serum from total protein extract showed positive titers to 1:8 while the serum from fixed parasites showed positive titers to 1:64. In conclude that for *T. evansi*, serum obtained against fixed parasites is the best for IFA because it preserves the antigenic membrane proteins. At the moment were using the serums to test Western blot.

Key words: *trypanosoma evansi*, antibodies, indirect immunofluorescence microscopy

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