TITLE: HAALSTRA METHOD ADAPTATION FOR THE ISOLATION OF *Dermatophilus congolensis*

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ABSTRACT:

Dermatophilosis is a zoonotic exudative dermatitis promoted by *Dermatophilus congolensis*, associated with the formation of crusts presenting an inferior concavity. This is caused by the superposition of epidermal cells together with the infectious agent and neutrophils. The diagnosis comprises the microscopic observation of the agent and the isolation by the Haalstra technique, which is based on the mobile zoospores chemotactism to CO_2. Traditionally in the technique, the crusts are fixed the bottom of tubes with distilled water, placed in an anaerobic jar with a CO_2 source, where they remain for 3 hours. Aliquots removed from the surface of the liquid are inoculated in an enriched culture medium. In this work, we aimed to isolate *Dermatophilus congolensis*, using an adaptation of the Haalstra method. Positive samples were triturated with 0.5 mL of sterile distilled water. The volume was fractionated into two parts and for each of them, 10mL of sterile H_2O was added. The first material was placed on a magnetic stirrer for 15 minutes and the second was centrifuged for 15 minutes at 3500 RPM. It was thus avoided to fix the material to the bottom of a tube, using some artifact, according to the original method. Both materials were kept at 25ºC for 2 hours and then placed in the GasPak System (BBL®). One drop of the supernatant from each sample was seeded in Petri dishes with Brain and Heart Infusion Agar and Sheep Blood Agar 5% (ASC5%) incubated at 35°C for 4 days. Centrifugation was shown to be effective in ASC5% culture after 4 days of incubation. The maceration followed by centrifugation promoted total immersion of the material in distilled water. This fact increased the motility of the zoospores, releasing them from crusts and migrating to the surface, which optimizes the isolation. It also reduces the contaminants, since most of these micro-organisms are devoid of flagella and without tropism to CO_2. The reduction of the resting time of the sample to two hours after the centrifugation was enough to obtain the colonies. The method using a magnetic stirrer for deposition of the samples and, consequently, of the microorganisms, was not effective in obtaining pure colonies. It is concluded that the use of sample centrifugation to release zoospores and maintenance of crusts and non-mobile contaminating microorganisms at the bottom of the containers for 2 hours period is efficient for the isolation of *D. congolensis*.

Keywords: *Dermatophilus congolensis*, Diagnosis, Haalstra

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