Acridine Orange staining for quick detection of blood parasites

College of Veterinary and Animal Sciences, Pookot, Wayanad-673 576, India

Abstract

Acridine Orange staining technique was found to be accurate, simple and rapid for screening large number of blood smears for the detection of haemoprotozoans and rickettsiales. Low parasitaemia can be detected with better accuracy.

Keywords: Acridine Orange, Staining, Blood parasites.

Introduction

Microscopic examination of Giemsa stained blood smears is the 'gold standard' for diagnosis of many haemoproteozoan and haemorickettsial infections. But, Acridine Orange staining enables more rapid detection and identification of blood parasites. It is a fluorochrome stain, which gives a red fluorescence of ribonucleic acid (RNA) and a green fluorescence of deoxy ribonucleic acid (DNA) (Hansen et al., 1970). This technique offers a higher sensitivity compared to Giemsa staining (10^-7 i.e. one parasite per 10^7 erythrocytes compared to 10^-2 or 10^-3) in the detection of Babesia sp. (Bose et al., 1995).

Materials and Methods

Acridine Orange (0.01%) stain was prepared by adding 20 mg AO powder (Himedia Laboratories) to 190 ml sodium acetate buffer. Stock solution of the buffer was prepared by adding 13.6 g of sodium acetate to 100 ml of distilled water and 90 ml of 1N HCl. The final pH was adjusted to 3.5 by adding 1N HCl.

Thin peripheral blood smears of 46 dogs and 32 bovine calves previously detected positive for Ehrlichia canis and Babesia bigemina, respectively were prepared and fixed in methanol. They were stained with AO (Lauer et al., 1981). The methanol fixed smears were flooded with 0.01% AO stain, allowed to act for two minutes and then washed slowly in tap water. The smears were mounted with a coverslip and examined when moist, under a fluorescent microscope. A drop of glycerol saline (1:1) was applied over the coverslip before examination under 100X magnification.

Fig. 1. Acridine Orange stained blood smear showing Babesia bigemina piroplasms

Fig. 2. AO stained blood smear of dog showing E. canis morulae inside monocyte
Results and Discussion

Acridine Orange staining of thin blood films prepared from calves showing parasitaemia, revealed yellowish coloured piroplasms of *B. bigemina* (Fig. 1). Bright orange coloured morulae of *E. canis* (Fig. 2) were clearly discernible within the cytoplasm of monocytes, which could be well differentiated from greenish coloured leucocytic nuclei. Gainer (1961) had observed *Anaplasma marginale*, a rickettsial organism as brilliant orange spots in the erythrocytes. Dutta *et al.* (1985) had demonstrated *Ehrlichia* sp. organisms in mouse macrophage cell cultures by Acridine Orange staining. Sreekumar (1992) observed yellow coloured *E. bovis* morulae with AO staining.

Total time required for staining and examination of the smears were less than 5 minutes. The technique was found to be accurate, simple and rapid for screening large number of blood smears for the detection of blood parasites. One advantage with the AO technique is that the smears stained by this technique can be restained with Romanowsky dyes, but only unstained preparations can be stained by AO dye (Hansen *et al.*, 1970).

References


