RAPID QUANTITATIVE ASSESSMENT OF THEILERIA INFECTION IN Ticks

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SUMMARY

A simplified method for methyl green pyronin staining is described for Theileria parva and T. annulata in whole salivary glands of Rhipicephalus appendiculatus and Hyalomma anatolicum subspecies respectively. The stain gives results comparable with Feulgen staining and can be used after the ticks have been in cold storage for 3 days. There is considerable variability in the rate and intensity of infection of these ticks with theilerial parasites and it is concluded that the method permits large samples (60 ticks per person per day) to be examined to overcome this variability when assessing infection quantitatively.

INTRODUCTION

In studies on the transmission of East Coast fever and tropical theileriasis it is useful to be able to assess rapidly and accurately the infection rates of the vector ticks with T. parva and T. annulata respectively. Blewett and Branagan (1973) developed a novel method for the detection of the parasites in whole salivary glands by Feulgen staining which gives excellent quantitative results. Subsequently the use of methyl green and pyronin stains for this purpose by Batungbacal (1974), Amure (1975) and Martins (1977) indicated that this approach could be adapted to a simpler stain. This paper describes the simplification of methyl green pyronin staining methods to make them rapid enough for examining large samples of ticks.

METHODS

Maturation of the parasite

The method relies on the demonstration of large masses of infective particles (sporozoites) of the parasite which mature in the acini of the salivary glands of adult ticks during feeding. Males and females of R. appendiculatus, H. anatolicum anatolicum and H. anatolicum excavatum are applied to rabbits' ears in cloth bags (Irvin and Brocklesby, 1970; Srivastava and Sharma, 1977). Ticks unnattached after one day are removed and those continuing to feed are left until day 3 after application (day of application counted as day zero) then removed for staining (Purnell and Joyner, 1968). Attachment is sometimes poor and this makes standardisation of the partial feeding difficult, since if all the ticks in a poorly feeding test batch have to be used this will increase the variability of the results.

Staining

The ticks are mounted in wax in a dish and the dorsum and all viscera except the salivary glands are removed. The salivary glands are freed from the posterior tracheae and anterior salivary duct with forceps and transferred to a staining tray. This tray is constructed from stainless steel wire mesh (20 meshes/cm, 0.3 mm pore diameter) and epoxy resin glue and it is bent into 20 compartments approximately 15 x 10 mm square and 10 mm deep. The tray stands in ethanol fixative and 20 pairs of glands can be accumulated per tray. Treatment is as follows:
1. Fix in 100% ethanol\(^1\) for a minimum of 10 min.
2. Stain directly in methyl green pyronin for 30 min.
3. Wash directly in 100% ethanol\(^1\) for 2 min.
4. Dehydrate in 100% ethanol for 5 min.
5. Clear in xylol for a minimum of 5 min.
6. Tease out on a slide in a drop of thin synthetic resin mountant.\(^2\)
7. Mount directly with a coverslip.

The stain is prepared by a modification of the method of Trevan and Sharrock (1951) as follows. A 2 % aqueous solution of methyl green (Gurr) is washed in chloroform until the chloroform remains clear. A 5 % aqueous solution of pyronin Y (Gurr) is prepared. These stock solutions are stable and fresh stain is made up with 10 ml methyl green stock and 1 ml pyronin stock in 250 ml of distilled water. A 0.2 M acetate buffer solution of pH 4.8 is made up from 119 ml of 0.1 M sodium acetate with 81 ml of 0.1 M acetic acid. The working solution is made up fresh with equal volumes of the stain solution and buffer solution.

The mounted glands can be examined immediately under a stereoscopic microscope at approximately ×50 magnification with intense transmitted light. The total number of acini containing parasite masses is then easily counted.

RESULTS

Appearance of staining

The nuclei of the acinar cells stain a blue/green colour with methyl green and the cytoplasm should be clear. Parasite masses of *T. parva* stain the same blue/green colour as the acinar cell nuclei when both immature and mature and detection of the parasite masses is by their size and shape as shown in Fig. 1. The nuclear material of the parasite masses is diffuse which diffuses the overall staining effect but parasitised acinar cells are also distinguished by their hyperplastic nuclei which stain intensely.

The parasite masses of *T. annulata* also stain the same blue/green colour when mature but the immature forms stain variably with the red pyronin and it is for this reason that the pyronin is included in the stock stain. The mature parasite masses of *T. annulata* are larger than those of *T. parva* as shown in Fig. 2.

Comparison with Feulgen staining

A batch of adult *R. appendiculatus* which detached as engorged nymphs on the same day from a calf infected with *T. parva* (Muguga strain) was fed on rabbits for 3 to 4 days and the salivary glands were removed and stained. Of each pair of glands one was stained in methyl green pyronin and the other with Feulgen as described by Blewett and Branagan (1973). Of 65 ticks, 25 were positive in both glands and the results are analysed further in Table I. From comparisons of the morphology and distribution of positive acini it is assumed that the masses staining with methyl green pyronin are theilerial parasite masses; ticks from the same batch were successfully used to infect tissue culture material with *T. parva* and no such masses have been found in uninfected batches of ticks. The methyl green pyronin method appeared in this small sample to be at least as sensitive as the Feulgen method.

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\(^1\) Industrial methylated spirit can be substituted for fixation and washing.

\(^2\) Depex (Gurr) diluted with xylol is currently used.