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The value of in vitro cell culture of granulocytes in the detection of Ehrlichia

Valor da cultura celular de granulócito *in vitro* na detecção de *Ehrlichia*

Alex Mutani[1] and James Stwart Kaminjolo[1]

Abstract *Peripheral blood leukocytes from different animals were isolated from whole blood and maintained in Dulbeco's medium containing homologous serum without antibiotics. After 72 hrs microscopic examination of these cells showed that most animals were infected with* Ehrlichia*. Observation of thin blood smears from the same animals showed that only two were positive for* Ehrlichia*. The results of this investigation show that leukocyte culture is superior to the traditional thin blood film method in the detection of* Ehrlichia *and that asymptomatic carriers are easily detected. The method is inexpensive and does not require specific cell lines although it is necessary to use sterile sera.*

Key-words: Ehrlichia*. Cell Culture. Mononuclear cells.*

Resumo *Leucócitos do sangue periférico de diferentes animais foram isolados do sangue total e mantidos em meio de Dulbeco, contendo soro homólogo sem antibióticos. Após 72 horas, um exame microscópico destas células mostrou que a maioria dos animais era infectada com* Ehrlichia*. Observação de esfregaços de sangue dos mesmos animais mostrou que apenas dois eram positivos para* Ehrlichia*. Os resultados desta pesquisa mostraram que a cultura de leucócitos é superior ao método tradicional de película de sangue na detecção de* Ehrlichia*, e que portadores assintomáticos são facilmente detectados. O método é de baixo custo e não exige linhas de células específicas, embora seja necessário o uso de soro estéril.*

Palavras-chaves: Ehrlichia. Cultura celular. Células mononucleares.

Natural infections of *Ehrlichia* pose diagnostic problems. In many cases the agents can neither be demonstrated in capillary blood films nor can they be detected in tissue impressions[11]. For many years, diagnosis of ehrlichiosis depended on clinical signs. This method has considerable limitations since several other pathogens may produce similar findings. Detection of the organism in blood films is a time consuming and laborious process, often resulting in false negatives[6]. Although serology has been utilized in the diagnosis of ehrlichiosis, it is often associated with problems such as strain variations[5] and inter-specific cross-reactivity[8 12]. Belongia *et al*[1] showed that serological methods may produce either false negatives due to use of acute sera or false positives from convalescent sera. The polymerase chain reaction is now a method of choice for diagnosis of ehrlichiosis because of its high predictive value[9 10]. Unfortunately this method is associated with economic constraints which, in many laboratories with limited resources, would be prohibitive. Since the development of an *in vitro* method for the cultivation of *E. canis* by Nyindo *et al*[14] similar reports have been produced by other workers[10 11 13 17]. In all the published reports some pre-requisites for the successful establishment of *Ehrlichia in vitro* are mentioned. These include the stage of infection of the animal, whether acute or chronic[15], the presence of specific cell lines[10 11 12] or the requirement of repeated peritoneal lavage to obtain infected cells[17]. The results of the present investigation show that:

A) in vitro culture of *Ehrlichia* can be carried out using only the individual host's peripheral blood leukocytes, hence eliminating the requirement for specific cell lines.

B) this method can detect asymptomatic carriers.

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MATERIAL AND METHODS

Thin blood smears were prepared from six dogs, ten cattle, two horses, and one goat. The animals were of different ages and originated from different areas in Trinidad. Their previous exposure to ixodid ticks was not available. Apart from the dogs, which were brought to the veterinary clinic for various reasons, all other animals were apparently healthy (Table 1). The smears were stained with Wrights-Giemsa stain and examined under immersion oil. From each animal 7.5ml of blood was drawn aseptically into sterile *monoject* heparinized tubes (Sherwood Medical, St. Louis, Missouri, U.S.A.). The tubes were left in a vertical position at room temperature for various times depending on the erythrocyte sedimentation rate of the species (canine: 1 h.; bovine: 24 hr.; equine: 30 m. and caprine: 24 hr.) as reported by Benjamin[2]. Thereafter, the plasma and leukocytes were aseptically collected into Leighton tubes, allowing 2ml per tube. The tubes were incubated at 37°C for 48hr under atmospheric conditions. The plasma was then discarded and the tubes washed with Dulbeco's medium without antibiotics. Two mililitres of Dulbeco's medium containing 20% homologous serum without antibiotics was then added to each tube. The tubes were subsequently fed with the same medium between 48 and 72 hrs. At the end of two and four days post-incubation the cover slips were removed, stained with Wrights-Giemsa stain and examined under immersion oil under a Nikon Optiphot-2 photomicroscope.

Table 1 - Species and clinical status of animals tested.

Species	Nr. tested	Clinical status	Nr. positive in using	
			blood slide	culture
Canine	6	asymptomatic	1	6
Bovine	10	asymptomatic	1	10
Equine	2	asymptomatic	0	2
Caprine	1	asymptomatic	0	1

RESULTS

Figure 1 shows the appearance of ehrlichial organisms as seen in bovine peripheral blood smear. Although blood smears from some animals were negative, (Table 1) results from cell culture revealed that all animals tested were infected with *Ehrlichia*. The organisms could be detected in mononuclear cells as early as 2 days post-incubation. Initially the organisms appeared as small and monomorphic bodies which changed into polymorphic bodies by the fourth day post-incubation (Figures 2 and 3).

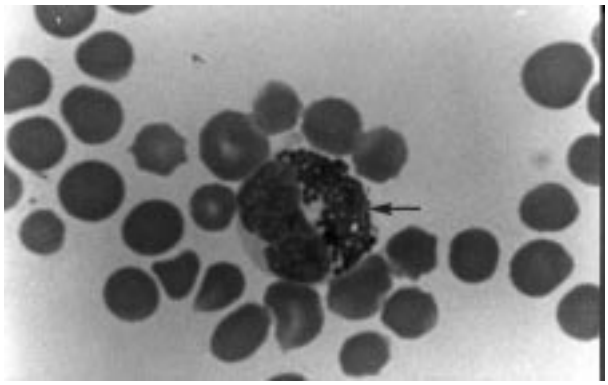

Figure 1 - Bovine peripheral blood mononuclear cell showing monomorphic inclusions (è) -Wrights-Giemsa stain x 1000.

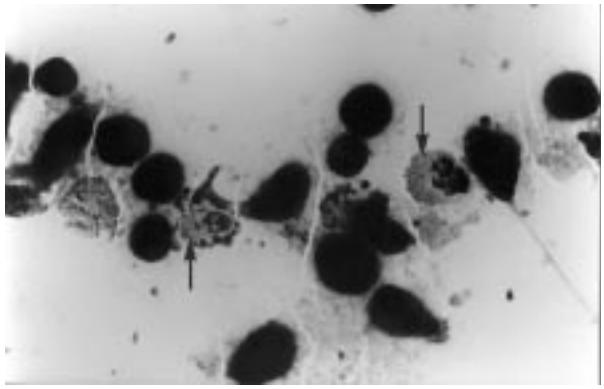

Figure 2 - Equine mononuclear cells at day 4 post incubation showing polymorphic bodies (è) -Wrights-Giemsa stain x 1000.

Figure 4 shows a canine mononuclear granulocyte on the fourth day of culture. Several bodies are evident, displacing the host cell's nucleus to the periphery. After five days of incubation most of the host cells had disintegrated and several bodies could be seen in the extracellular space (Figure 5).

DISCUSSION

The morphological appearance of the organisms both in peripheral blood smears and in culture conformed to those originally reported by Ristic and Huxsoll[16]. In the present investigation it was noted that blood smears from the majority of animals tested were negative; only becoming positive after culture. These results suggest that parasitaemias in the originally *negative* animals were too low to be detected by the usual blood smear

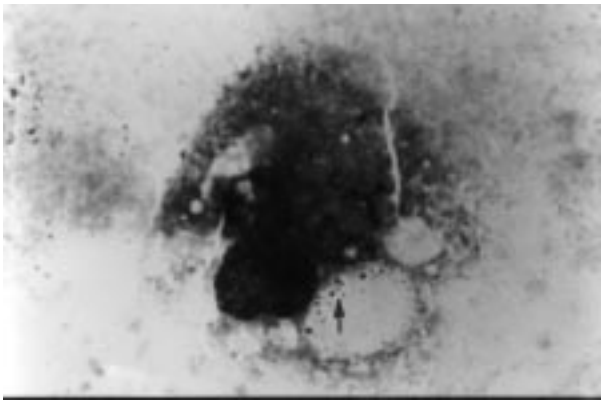

Figure 3 - Caprine mononuclear cell at day 4 post incubation showing polymorphic bodies intra and extracellulary (è) - Wrights-Giemsa stain x 1000.

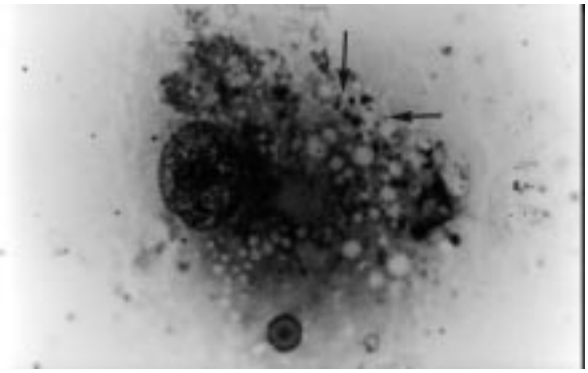

Figure 4 - Canine mononuclear cell at day 4 post incubation showing polymorphic cellular inclusions (è) - Wrights-Giemsa stain x 1000.

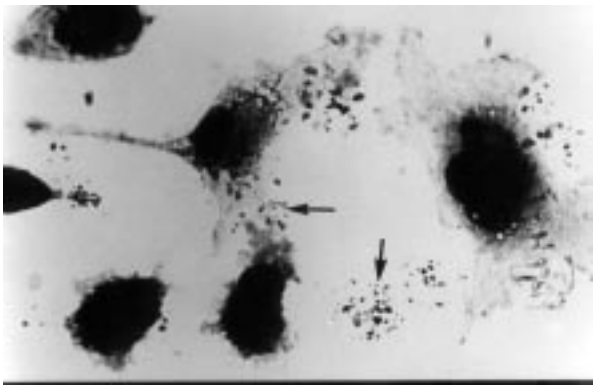

Figure 5 - Bovine mononuclear cells at day 5 post incubation showing several extracellular polymorphic bodies (è) amongst host cell debris Wrights-Giemsa x 1000.

techniques. These observations support those of Dawson *et al*[6]. In addition Carmichael and Fiennes[3] and Carter *et al*[4] reported that in low parasitaemias, small ehrlichial bodies and azurophilic granules could be confused with each other, thus contributing to the difficulty in microscopic diagnosis using blood smears. The present results show that cell culture amplifies the numbers of ehrlichial bodies in a reasonably short time and do not reveal the species identity of the ehrlichial organisms. However, these results suggest that *Ehrlichia* may be widespread in many animal species in Trinidad. Several species of *Ehrlichia* are known to exist[16]; being transmitted by various species of ticks present in a particular locality. In Trinidad and Tobago tick genera which have been reported include *Amblyomma*, *Anocentor*, *Boophilus*, *Haemaphysalis*, *Ixodes* and *Rhipicephalus*[7]. Although ticks may cross host species, the question whether *Ehrlichia* can cross host species remains to be resolved. The present results have shown that many apparently healthy animals may be carriers of *Ehrlichia* and that these asymptomatic cases can be detected by a relatively simple culture technique.

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