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A haematocrit centrifuge concentration technique for the diagnosis of bovine babesiosis due to *Babesia bigemina* infection.

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Keywords: *Babesia bigemina* — Parasitological diagnosis — Chronic bovine babesiosis.

Summary

It is well known that the chronic form of bovine babesiosis due to *B. bigemina* infection is usually characterized by a low grade parasitaemia and that the thin blood smear examination is often ineffective for parasitological diagnosis.

For detecting this form of infection, a haematocrit concentration technique is described. This technique, based on the density and the tropism of *B. bigemina* for young erythrocytes, is very efficient and easy to use in the field conditions. The method was evaluated in experimentally and naturally infected cattle. It increases the level of detection of infection up to five times in the chronic phase of the disease.

Résumé

Il est bien connu que la forme chronique de la babésiose bovine due à *B. bigemina* est caractérisée par une faible parasitémie que souvent l'examen des frottis de sang ne permet pas de détecter.

Pour détecter cette forme de babésiose une technique de concentration par la méthode de l'hématocrite est décrite. Cette méthode est basée sur la densité des érythrocytes et le tropisme de *B. bigemina* pour les érythrocytes immatures. Les résultats de son utilisation dans le diagnostic des infections expérimentales et naturelles sont rapportés. Cette méthode augmente plus de cinq fois la mise en évidence des parasites dans la phase chronique de la maladie.

Introduction

Babesia spp. are intra-erythrocytic sporozoa. Babesiosis is a tick-borne disease which constitutes a very important constraint for animal health and production (8, 9).

Depending on the susceptibility of the host and the virulence of *B. bigemina* strains, cattle may develop acute, subacute, chronic or asymptomatic babesiosis. In the last two forms, parasitaemia is difficult to detect, nevertheless it constitutes a source of infection for the healthy stock (16).

For the diagnosis of chronic bovine babesiosis due to *B. bigemina* infection, thin as well as thick blood smear examinations are ineffective. Although the thick smear method has some advantages, the small quantity of blood used and the morphological damage of the parasite during the smear preparation limit its efficiency (2,7,14). Although serological techniques, especially the indirect immunofluorescent antibody test (IFAT) can reveal chronic babesia infections, they are not conclusive in demonstrating the presence of parasites.

Considering these limitations of the classical smear methods, a more sensitive and parasitological technique is required in order to diagnose early stages of infection in bovines and, from the epidemiological point of view, to detect low grade of parasitaemia which characterize chronic *B. bigemina* infection.

On the basis of certain well known features of the

erythrocytes, such as, low density of the young erythrocytes and the parasitized erythrocytes as well as the tropism of *B. bigemina* for young erythrocytes (1,9,11,15) a haematocrit centrifuge concentration technique is described. The procedure is evaluated on experimental and natural cases of bovine babesiosis.

Materials

Two calves (red Belgian breed), 3 and 12 months old were experimentally exposed to *B. bigemina*, Nigerian strain GU 177 stabilate, by intravenous inoculation. Furthermore, 312 six to 24 months old cattle (local breeds, European imported breeds and their cross-breeds), from South-Western Angola where bovine babesiosis due to *B. bigemina* is endemic were examined.

Technique

The haematocrit centrifuge concentration technique (HCT) was used and carried out as follows. Heparinized capillary tubes, 75 mm long and 1.2 mm in diameter were filled with 70 μ l of blood and centrifuged at 12,000 rpm 3 to 4 minutes (fig. 1.a).

The upper layer of 2 to 3 mm depth of the packed red blood cells was placed on the slide and mixed with an equal volume of plasma (fig. 1.c). The mixture was spread to make small thin and thick smear preparations on the same slide (fig. 1.d.).

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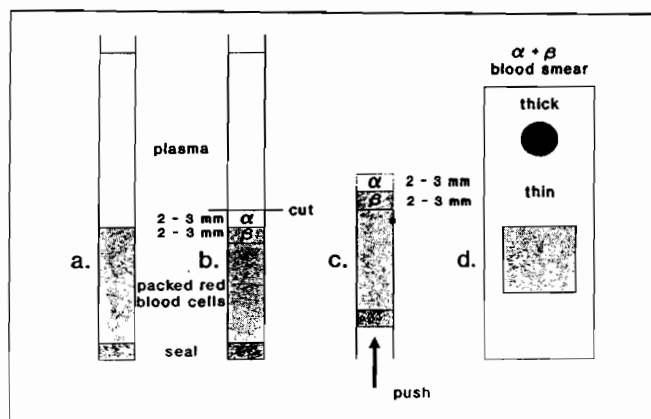


Figure 1

- a: capillary tube after centrifugation
 b: cut tube with diamond pen
 c: push out 4-6 mm of contents on a glass slide
 d: mix $\alpha + \beta$ for thick and thin blood smear

When dried these smears were methanol fixed and Giemsa stained. These preparations were examined for 15 minutes using oil immersion objective at $\times 400$ magnification. For comparison, the classical thin blood smears were made and also examined for 15 minutes.

Results

From the two experimentally infected calves one developed an acute disease and died while the other developed chronic babesiosis. In the two calves, the HCT demonstrated parasitaemia as early as 4 days after inoculation. On the 5th and 6th day after infection the HCT revealed 4.3% and 7.9% of RBC parasitized whereas with the thin blood smear examination, less than 1% RBC were shown to carry the infection. From 60th day after inoculation, only HCT revealed the infection.

When the 312 samples of field cases of Angolan bovines were examined, HCT demonstrated 73 (23.40%) parasitized animals as against 14 (4.49%) diagnosed by thin smear preparation (table 1).

TABLE 1

Comparison of TBS* and HCT** for the diagnosis of bovine babesiosis due to *Babesia bigemina*.

Results		Samples examined	
		number	%
TBS+	HCT+	12	3,85
TBS-	HCT-	237	75,96
TBS+	HCT-	2	0,64
TBS-	HCT+	61	19,55

* Thin blood smear (TBS)

** Haematocrit centrifuge concentration technique (HCT)

Discussion

Earlier, a centrifugation technique, using spin rate of 1,500 - 2,000 rpm and based on the difference in the density between parasitized and uninfected erythrocytes was described and used for the diagnosis of *B. caballi* infection in equines (10).

Results obtained with the haematocrit centrifuge concentration method on the experimental and natural cases of bovine babesiosis prove that this technique is feasible in revealing early infections and, in the field when compared with the thin blood smear method. It increases the level of detection of infections up to five times in the chronic phase of the disease.

Therefore, it appears that HCT is a quite sensitive parasitological method for the diagnosis of chronic form of *B. bigemina* infection. It is easy to perform in the field conditions using a minicentrifuge with electrical supply as well as a hand-powered microcentrifuge (4). Although HCT is less sensitive than IFAT, it is a useful method for confirmation of the infection by demonstrating the presence of the parasites in red blood cells.

For detecting low grade parasitaemia in chronic bovine babesiosis, the recent development of DNA probes provides a highly sensitive technology. This technique is not yet available for use on large scale and in the field conditions (3,5,6,10).

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